US EPA ARCHIVE DOCUMENT
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)

Through: Clint Skinner, Ph.D. Section Head
Review Section III
Toxicology Branch
HED (TS-769)

Theodore M. Farber, Ph.D., Chief
Toxicology Branch
HED (TS-769)

Compound: Triforine
Caswell No.: 890A
Registration No. 21137-3
Registrant: E. M. Industries
Accession No.: 253815

Act on Requested: Review studies (number RCC 026256 and RCC 021037) on Triforine: Mouse Micronucleus Assay as a replacement for the dominant lethal assay (IBT 622-5459) which has involved review filed by Mitre Corporation. (EPL).

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

Toxicologic Review

Compound: Triforine

Caswell Numbers: Caswell Number 890A

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 micronuclei.

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: Triflurine

Compound Number: Caswell No. 8984


Reviewed By: Stephanie P. Horii, Ph.D.
Section III
Toxicology Branch, NIEH

Secondary Review: Irving Reiner, Ph.D., Genentech Review Section VI
Toxicology Branch
HED/MbF

Core Classification: Inconsistent

Conclusion: As in the previous experiment as there were no effects on the target tissue 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: Triflurine HCl - 1.4
superseded by - 2.3 - (2,2,2 - trifluoroethanol) benzene - 3% (formica), batch lot number 1108. 98.6 sure claim.
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 Trifluron [N,N'-[1,4-piperazinediy]-bis-(2,2,2-trichloroethylidene)-bis-(formamide)], Lot number 1990, 98.8% pure (analysisumber ALH 2319312 v. 20.07.83) powder dissolved in 2% Carboxymethylcellulose 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.

3. The positive control group received 50 mg/kg body weight of cyclophosphamide (reference mutagen) dissolved in 0.9% saline solution.

Methods:

The mice were randomly assigned to three groups consisting of 16 males and 16 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 dosage volume of all materials was 20 ml/kg animal body weight.

The body cavities from both femurs were prepared for evaluation. The evaluation (Schmitz, K.) The micronucleus test, Mutat Res 31: 9-15, 1975) was based upon scoring 1000 erythrocytes polychromatnic (PC) and normochromatnic (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., 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/MCE ratios.
Background

There are no acceptable mutageniciry 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 mutagenicity assays to fill this data gap.