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OFFICE OF
PESTICIDES AND TOXIC SUBSTANCES

TOX Chem No.: 481C
HED Project No.: 9-2181
RD Record No.: 251,976

MEMORANDUM

SUBJECT: Triazine - Mutagenicity Data Submitted under MRID
Nos. 41231701 and 41231702
EPA ID No. C-62372

FROM: Irving Mauer, Ph.D., Geneticist *Irving Mauer 01-03-90*
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Health Effects Division (H7509C)

TO: James E. Wilson, PM Team 31
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THRU: Karl P. Baetcke, Ph.D., Chief *Karl P. Baetcke 1/13/90*
Toxicology Branch I - Insecticide-Rodenticide Support
Health Effects Division (H7509C)

Registrant: Triazine Joint Venture, c/o Lehn and Fink,
Montvale, NJ

Request

Review and evaluate the following mutagenicity studies,
both performed by Toxicol Labs, Ledburg, Herfordshire (UK):

1. Mouse Micronucleus Test: Triazine, Study No.
M/MMN/10659, Final Report dated February 3, 1989
(EPA MRID No. 41231701).

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2. Bacterial Reverse Mutation Assay: Triazine, Study No. M/Ames/10659, Final Report dated February 8, 1989 (EPA MRID No. 41231702).

TB Conclusions

(See appended detailed reviews.)

Study	Reported Results	TB Evaluation
1. Micronucleus	Negative for inducing micronuclei in bone marrow cells of CD-1 mice treated orally up to 855 mg/kg (80% of the LD ₅₀)	ACCEPTABLE
2. Ames	Negative for inducing increases in reverse gene mutation in Salmonella strains treated up to toxic levels (200 μ g/plate), +/- S9	ACCEPTABLE

Attachments (DERs)

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Reviewed By: Irving Mauer, Ph.D., Geneticist
Toxicology Branch I - IRS/HED (H7509C)
Secondary Reviewer: Karl F. Baetcke, Ph.D., Chief
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01-03-90
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DATA EVALUATION REPORT

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I. SUMMARY

MRID No.: 412317-01
ID No.: C-62372
RD Record No.: 251,976
Caswell No.: 481C
Project No.: 9-2181

Study Type: (84-2) Mutagenicity - Chromosome damage in vivo
(Mouse MT)

Chemical: Triazine [hexahydro-1,3,5-tris(hydroxyethyl)-
s-triazine]

Sponsor: Triazine Joint Venture
c/o Lehn and Fink Products
Montvale, NJ

Testing Facility: Toxicol Labs, Ledburg, Herfordshire (UK)

Title of Report: Mouse Micronucleus Test [On] Triazine.

Author: J.C. Asquith

Study No.: M/MMN/10659

Date of Issue: February 3, 1989

TB Conclusions:

Negative for inducing micronuclei in bone marrow cells
of CD-1 mice treated by gavage at single oral doses up to
855 mg/kg, representing 80 percent of the LD₅₀.

Classification (Core-Grade): ACCEPTABLE

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II. DETAILED REVIEW

A. Test Material - Triazine (from Lehn & Fink)

Description: Clear yellow liquid
Batch (Lot): (Not provided)
Purity (%): 78.5
Solvent/Carrier/Diluent: 0.9% (1N) sterile saline
(NaCl)

B. Test Organism - Rodent

Species: Mouse
Strains: CD-1
Age: 8 to 9 weeks
Weights - Males: 20 to 34 g
 Females: 24 to 31 g
Source: Charles River (UK), Maidstone, Kent

C. Study Design (Protocol) - This study was designed to assess the clastogenic potential (leading to the formation of PCE micronuclei) of triazine when administered by oral gavage to CD-1 mice. Statements affirming compliance with Agency GLPs as well as for Quality Assurance measures (inspections/audits) were provided in the Final Report.D. Procedures/Methods of Analysis - Following a range-finding study (at doses up to 2000 mg/kg), groups of CD-1 mice (5/sex/group) received single oral doses of triazine, and were sacrificed 24, 48, or 72 hours later. Control groups received 0.9% saline (solvent control), or cyclophosphamide (CPA, positive control), the latter sacrificed only at 24 hours. Femoral bone marrow harvested at scheduled sacrifice times was prepared for microscopic examination by conventional cytological techniques. At least 1000 polychromatic erythrocytes (PCE) per animal were scored for micronuclei (MN-PCE) on coded slides, and the ratio of PCE to normochromatic erythrocytes (NCE) also recorded for each animal.

The micronuclei data were analyzed by the Mann-Whitney U-test, with probabilities for significant differences between test and control groups obtained from standard published tables. All dosing solutions were analyzed for actual concentrations of triazine, in order to confirm that nominal levels of test article were delivered. [Spectrophotometric measurements revealed actual concentrations ranged from 98 to 113% of nominal.]

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- E. Results - In the range-finder test (Report Appendix 2), deaths occurred at 1200 and 2000 mg/kg (respectively, 2 of 3 animals and 3 of 3 of each sex), but no mortalities at 800 mg/kg or below, providing an estimated LD₅₀ of approximately 1000 mg/kg. Based upon the (OECD) convention of applying 80 percent of the LD₅₀ as the MTD for this type of study, the HDT for the main study was selected as 800 mg/kg, with two lower doses of 400 and 200 mg/kg.

[Individual animal data for the main assay were tabulated in Report Appendix 1, and summarized by group in Report Tables 1, 2, and 3, as well as in a Data Summary Form following Report Appendix 3.]

In the micronucleus assay itself, two high-dose (800 mg/kg) animals (one of each sex) died before their scheduled sacrifice at 48 hours. No cytotoxicity, as measured by reduction in PCE/NEC ratios, was found in any nabam-treated group. Random variation around control values for numbers of micronuclei was found at all sacrifice times and in both sexes of test animals. A singular statistically significant increase in micronuclei over concurrent control was calculated for the 400 mg/kg male group sacrificed at 72 hours (0.14 vs. 0.02 - see Table 3, attached). This increase was considered to be of no biological significance since it fell within solvent control ranges at other sampling times (notably 24 hours when optimally most of the micronuclei would be visualized).

Hence, the investigator concluded that triazine did not induce micronuclei in bone marrow cells of mice treated orally up to a nominal dose (800 mg/kg) considered a MTD by OECD convention (= 80% of the LD₅₀).

- F. TB Evaluation - ACCEPTABLE, as demonstrating no potential for the induction of micronuclei in CD-1 mice. This study was conducted in a manner consistent with adequate practices for this type of assay. Although the Agency's limit dose in the absence of any toxicity (=1000 mg/kg) was not assayed (represented by the LD₅₀ calculated in the range-finder), we believe testing up to 800 mg/kg is sufficiently high to validate the negative result obtained.

Attachment (Summary Data Table)

Toxicol Reference :- M/MMN/10659

Date Received :- 19/5/88

Sample Product Code :- Triazine

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Sample Description :- Clear yellow liquid

Container :- Delivered to Toxicol in white plastic dispensing container
 Received by internal transfer in a clear glass bottle with
 white screw cap

Starting Date :- 7/11/88

Experimental Work Completed :- 6/12/88

Treatment	Mean Frequency of micronucleus formation %								
	24 hours			48 hours			72 hours		
	M	F	Total	M	F	Total	M	F	Total
Solvent Control	0.10	0.14	0.12	0.0	0.08	0.04	0.02	0.0	0.01
Positive Control CPA 40mg/kg	1.31	1.61	1.46						
200mg/kg Triazine	0.18	0.04	0.11	0.02	0.0	0.01	0.04	0.01	0.03
400mg/kg Triazine	0.20	0.14	0.17	0.0	0.04	0.02	0.14	0.0	0.07
800mg/kg Triazine	0.10	0.02	0.06	0.03	0.07	0.05	0.02	0.0	0.01

Conclusion :-

Triazine has been found not to be a mutagen when tested by the micronucleus test in the bone marrow of CD1 mice dosed at concentrations up to 855.3mg/kg.

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Reviewed By: Irving Mauer, Ph.D., Geneticist
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Secondary Reviewer: Karl P. Baetcke, Ph.D., Chief
Toxicology Branch I - IRS (H7509C)

Irving Mauer
01-03-90

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DATA EVALUATION REPORT

I. SUMMARY

MRID No.: 41231702
ID No.: C-62372
RD Record No.: 251,976
Caswell No.: 481C
Project No.: 9-2181

Study Type: (84-2) Mutagenicity - Gene mutation in
bacteria (Salmonella - Ames)

Chemical: Triazine [hexahydro-1,3,5-tris(hydroxyethyl)-
s-triazine]

Sponsor: Triazine Joint Venture
c/o Lehn and Fink Products
Montvale, NJ

Testing Facility: Toxicol Labs, Ledburg, Herfordshire (UK)

Title of Report: Bacterial Reverse Mutation Assay [On]
Triazine.

Author: J.C. Asquith

Study No.: M/Ames/10658

Date of Issue: February 8, 1989

TB Conclusions:

Negative for inducing increases in reverse mutation in
the standard battery of Ames strains exposed to test compound
up to toxic levels (200 $\mu\text{g}/\text{plate}$), with and without metabolic
activation.

Classification (Core-Grade): ACCEPTABLE

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II. DETAILED REVIEW

A. Test Material - Triazine (from Lehn & Fink)

Description: Clear yellow liquid
 Batch (Lot): (Not provided)
 Purity (%): 78.5
 Solvent/Carrier/Diluent: Sterile deionized water (DW)

B. Test Organism - Bacteria

Species: Salmonella typhimurium
 Strains: TA1535, TA1537, TA1538, TA98, TA100
 (all his⁻)
 Source: (Not stated)

C. Study Design (Protocol) - This study was designed to assess the mutagenic potential of triazine when administered in vitro to Salmonella (Ames) strains. Statements affirming compliance with Agency GLPs as well as with Quality Assurance measures (inspections/audits) were provided in the Final Report.D. Procedures/Methods of Analysis - Following range-finding toxicity testing with TA98 (only) at doses up to 5000 ug/plate, triplicate cultures of all five Ames strains were exposed for 72 hours^{1/} to test substance at each of five dose levels, both in the absence and presence of a mammalian metabolic activation system consisting of the liver microsomal fraction (S9) prepared from male Fischer 344 rats induced by Aroclor 1254 and containing 44 mg protein per mL, plus generating cofactors, as detailed in Report Appendix 3.

In addition to solvent controls (DW), bacterial strains were exposed to their selectively appropriate active mutagens in the absence of activation^{2/}, but all to 2-aminoanthracene (2-AA, 2 ug/plate) under activation, thus serving as positive controls. The entire experiment was repeated.

The number of revertent colonies per plate was counted electronically (Biotran III), treatment group means and standard deviations calculated, and resulting data statistically analyzed by ANOVA (F-statistic). If F

1/Extended from the customary 48 hours because triazine is bacteriostatic (thus slowing growth of revertent colonies).

2/Sodium azide (SA, 1 ug/plate) for TA1535 and TA100; 9-aminoacridine (9-AA, 50 ug/plate) for TA1537; 2-nitrofluorene (2-NF, 0.5 ug/plate) for TA1538 and TA98.

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was significant ($p < 0.05$) and dose-responsiveness apparent, a correlation coefficient was calculated over the response range, and its significance read from standard (published) tables. This laboratory considers a substance positive if it produces a reproducibly statistically significant increase in the mean number of revertents that exceeds twice the concurrent solvent control, plus evidence for a dose-response. Plates showing evidence of severe toxicity (markedly reduced or absent background bacterial lawn) were excluded from analysis, since these may yield significantly decreased revertent colony counts (of no relevance to mutagenic activity), or increased colonies from surviving non-revertant cells incorporating the histidine available because of (toxic) lysis (i.e., a false-positive result).

- E. Results - In the preliminary toxicity test, triazine was toxic to both non-activated and activated TA98 cells at dose levels of 200 $\mu\text{g}/\text{plate}$ and above, as revealed by reduced background lawn indicative of restricted growth. Hence, the five doses selected for the mutation assays were 200, 40, 8, 1.6, and 0.32 $\mu\text{g}/\text{plate}$ with or without S9 mix.

Individual plate colony counts for the two assays were provided in Report Appendices 1 and 2, summarized and analyzed in Report Tables 1 and 2, and the entire study presented in Data Summary Form following Appendix 3.

In both experiments, the test substance consistently reduced background lawns at the HDT, 200 $\mu\text{g}/\text{mL}$, in the absence of activation but with one exception did not induce significantly increased revertent colony counts. The exception was a significant F-value ($p < 0.05$) calculated for unactivated TA1537 cultures, derived from a wider-than-usual variation in mean numbers of revertents on test plates (3.3 to 7.7) compared to the concurrent mean control value (4.7). This was discounted by the investigator because there was neither a doubling of revertent rate nor evidence of a dose-relationship. Hence, the investigator concluded that triazine was not mutagenic in the Ames testing at levels up to levels of toxicity.

- F. TB Evaluation - ACCEPTABLE. The study was well conducted with adequate procedures under controlled conditions such that the negative results for triazine in repeat experiments represent valid interpretations of the data.

Attachments (Summary Data Table :)

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Toxicol reference :- M/AMES/10658

Date received :- 19/5/88

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Sample product code :- Triazine

Sample description :- Clear yellow liquid

Container :- White plastic dispensing container

Starting date :- 20/10/88

Experimental work completed :- 6/11/88

Dose range of product used :- 0 - 200µg/plate

Mutation Frequency in revertants/µg
from 2 experiments in strains ;

Strain	TA1535	TA1537	TA1538	TA98	TA100
Level of S-9 in mix	0%	0	0	0	0
	10%	0	0	0	0
Spontaneous revertants on control plates	10.3 ±1.87	8.7 ±3.57	14.6 ±6.40	20.9 ±5.61	118.7 ±8.32

Conclusion :-

Triazine is not mutagenic in the Ames test against the above indicator strains, when tested with and without metabolic activation, up to a toxicity limit of 200µg/plate.

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