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

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JUN 20 1990

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OFFICE OF
PESTICIDES AND TOXIC
SUBSTANCES

MEMORANDUM

SUBJECT: Simazine - Mutagenicity Data Submitted under MRID Nos. 414429-01 and -02
EPA ID #100-541

Chemical (Caswell) No.: 740
RD Record No.: 262,860
HED Project No.: 0-1092

FROM: Irving Mauer, Ph.D., Geneticist
Toxicology Branch I
Health Effects Division (H7509C)

TO: Jane M. Talarico, PM 74
Reregistration Branch
Special Review and Reregistration Division (H7508C)

THRU: Karl P. Baetcke, Ph.D., Chief
Toxicology Branch I
Health Effects Division (H7509C)
6/18/90

Registrant: Ciba-Geigy Corporation, Greenboro, NC

Request

Review and evaluate the following (two) mutagenicity studies, submitted as additional data for the reregistration of simazine, and both performed in the genetic toxicology laboratories of Ciba-Geigy Ltd., Basle (Switzerland):

1. Simazine Technical: Structural Chromosomal Aberration Test - Mouse Micronucleus Test (MT),

Lab. Study No. 881189, Final Report dated September 15, 1988 (EPA MRID 41442901).

2. Simazine Technical: Tests for Other Genotoxic Effects - Autoradiographic DNA Repair Test on Rat Hepatocytes (DNA), Lab Study No. 891412, Final Report dated December 7, 1989. (EPA MRID No. 41442902).

TB Conclusions:

[Detailed reviews are appended to this memorandum].

Study	Reported Results	TB Evaluation
(1) MT	Negative for inducing micronuclei in bone marrow cells of mice treated by acute oral gavage up to 5000 mg/kg.	ACCEPTABLE
(2) DNA	Although reported as negative in repeat assays at levels up to 170 ug/ml, no evidence was presented that a cytotoxic or solubility limit was tested.	UNACCEPTABLE

ATTACHMENTS (DERs)

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

6/18/90

DATA EVALUATION REPORT

I. SUMMARY

MRID (ACC) No.: 41442901
ID No.: 100-541
RD Record No.: 262,860
Caswell No.: 740
Project No.: 0-1092

Study Type: Mutagenicity - Chromosomal aberrations in vivo (micronucleus)

Chemical: Simazine

Synonyms: G-27-692 Technical

Sponsor: Ciba-Geigy, Basle

Testing Facility: Ciba-Geigy, Basle

Title of Report: Structural Chromosomal Aberration Test - Micronucleus Test, Mouse

Author: Carla Ceresa

Study No.: 88189

Date of Issue: September 15, 1988

TB Conclusions:

Negative for inducing micronuclei in bone marrow cells of mice treated by acute oral gavage up to 5000 mg/kg.

Classification (Core-Grade): ACCEPTABLE

II. DETAILED REVIEW

A. Test Material - G-27-692 technical (simazine)

Description: (Not stated)
Batch (Lot): 209158
Purity (%): 99.6
Solvent/carrier/diluent: Carboxymethylcellulose, 0.5% (CMC)

B. Test Organism - Rodent

Species: Mouse
Strain: Tif: MAG F/SPF (NMRI-derived)
Age: (Adult)
Weights - males: 27 to 38 g
females: 22 to 33 g
Source: Ciba-Geigy Tierfarm, Sisseln (Switzerland)

C. Study Design (Protocol) - This study was designed to assess the clastogenic potential of simazine when administered by oral gavage to mice, according to internationally-accepted test guidelines (OECD/EEC/EPA).

A statement affirming compliance with Agency GLPs was provided.

A Statement of Quality Assurance measures (inspection/audits) was also provided.

D. Procedures/Methods of Analysis - Following preliminary toxicity testing, the test article was administered by oral gavage to groups of male and female mice according to two schedules: (i) once at 5000 mg/kg, and animals sacrificed 16, 24, and 48 hours later; (ii) once, at doses of 1250, 2500, and 5000 mg/kg, and animals sacrificed 24 hours later. In addition to concurrent solvent controls for each one of the sacrifice times (0.5% CMC), groups of 8 males: 8 females received the clastogen, cyclophosphamide (CP, 64 mg/kg) and were sacrificed 24 hours later.

At the scheduled sacrifice times, bone marrow was collected from both femurs of each animal, and prepared for microscopic examination by conventional cytological techniques. Coded slides from 5 animals/sex/experimental group were examined under oil-immersion, and 1000 polychromatic erythrocytes (PCE) per animal scored for the presence of micronuclei; in addition, ratios of PCE to normochromatic erythrocytes (NCE) were determined for each animal.

The data were analyzed by Chi-square, with the level of significance set at $p \leq 0.05$.

E. Results - In neither experiment were any significant differences in incidences of micronuclei between simazine-treated (0.02 to 0.08%) and solvent control (0.06 to 0.08%) animals recorded (Report Table 2 to 8, appended to this DER). By contrast, highly significant increases ($p < 0.05$) were registered in both CP-treated positive controls (1.81 and 2.02%). No cytotoxicity by simazine treatment was found in bone marrow cells, since the ratios of PCE to NCE were unaffected (Tables 2 to 8); finally, no clinical effects of treatment were reported.

F. TR Evaluation - Acceptable. This study appears to have been properly conducted according to current testing guidelines under conditions assuring appropriate testing of the test substance for clastogenic potential when administered acutely by oral gavage up to the limit dose (5000 mg/kg).

ATTACHMENTS (Data Tables)

ATTACHMENT I

Data Tables

SIMAZINE

Page_____ is not included in this copy.

Pages 7 through 15 are not included.

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____ Identity of product inert ingredients.

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____ A draft product label.

____ The product confidential statement of formula.

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

6/18/90

007992

DATA EVALUATION REPORT

I. SUMMARY

MRID (ACC) No.: 41442902
ID No.: 100-541
RD Record No.: 262,860
Caswell No.: 740
Project No.: 0-1092

Study Type: Mutagenicity - DNA damage/repair in vitro (HPC/UDS)

Chemical: Cimazine

Synonyms: G-27-692 Technical

Sponsor: Ciba-Geigy

Testing Facility: Ciba-Geigy, Basle (Switzerland)

Title of Report: Tests for Other Genototoxic Effects - Auto-radiographic DNA Repair Test on Rat Hepatocytes.

Author: Thomas Hertner

Study No.: 891412

Date of Issue: December 7, 1989

TB Conclusions:

Although reported as negative for inducing unscheduled DNA synthesis (UDS) in rat hepatocytes treated up to 170 ug/mL, no evidence was presented that this represented a cytotoxic or solubility limiting concentration.

Classification (Core-Grade): UNACCEPTABLE

II. DETAILED REVIEW

A. Test Material - G-27-692 technical (simazine)

Description: (Not stated)
Batch (Lot): PL-850614
Purity (%): 96.9
Solvent/carrier/diluent: Dimethylsulfoxide (DMSO)

B. Test Organism - Rodent hepatocytes

Species: Rat
Strain: Tif:RAIF (SPF)
Age: (Adult)
Weights - males (only): 170 to 350 g
Source: Ciba-Geigy Tierform, Sisseln (Switzerland)

C. Study Design (Protocol) - This study was designed to assess the DNA-damaging potential of simazine as determined by unscheduled DNA synthesis when administered in vivo to primary rat hepatocyte cultures, according to internationally accepted test guidelines (OECD, EEC, EPA).

A statement affirming compliance with Agency GLPs was provided.

A Statement of Quality Assurance measures (inspections/ audits) was also provided.

D. Procedures/Methods of Analysis - Hepatocytes were isolated from adult male rats by established procedures, and allowed to attach to coverslips immersed in multi-well culture vessels containing appropriate culture medium. Following preliminary cytotoxicity testing for dose selection, cultures of attached cells are treated in quadruplicate for 16 to 18 hours with test substance (at least 6 preselected concentrations), or with vehicle (DMSO), or with the mutagen 2-acetylaminofluorene (AAF, 45 uM); as well, untreated (culture medium only) negative controls were run concurrently.

All cultures were treated concurrently with tritiated thymidine (8 u Ci ^{3}H-TdR, spec. act. = 25 C/mmol) following which the cells were prepared for autoradiography with Ilford K-5 photographic emulsion on microscope slides (under darkroom conditions) by standard procedures. Slides were then placed in air- and light-tight boxes at 4 °C for 4 days, following which slides were stained (hemotoxylin-eosin) and made permanent.

Developed silver grains were counted over the nuclei and cytoplasm of 50 cells per slide (150 per treatment), by means of an electronic counter attached to a universal binocular microscope (magnification = 2000X). Net grain counts were calculated by subtracting the average grain count over three nuclear-sized cytoplasmic areas from the nuclear grain count, and all calculations summarized for each treatment.

Grain count data were analyzed statistically by Dunnetts one-tailed t-test, with significance set at $p \leq 0.01$. The following criteria were applied for determining the quality of response:

For a positive:

"The mean gross number and the mean net number of silver grains per nucleus in relation to their respective vehicle controls are significantly different at any concentration, and the mean net value is at least 2.0."

For a negative:

"The mean gross number and the mean number of silver grains per nucleus in relation to the vehicle control are not statistically different at any concentration and no concentration dependence can be seen."

The entire assay was repeated once ("confirmatory test").

E. Results - In the preliminary toxicity testing, the highest concentration considered "usable"* was 170 ug/mL (Report Table 1, appended here).

In the initial repair test carried out at doses of 1.57, 4.72, 14.17, 42.50, 85, and 170 ug/mL, none of the mean gross or net grain counts in treated cultures were statistically different from vehicle controls (Report Table 3, 7, 8, and 9, appended here). However, in comparison with the medium control, a slight shift to higher distribution values was noted for the three highest concentrations (Table 4, attached). In the confirmatory trial (using the same dose schedule), none of the grain values in simazine-treated cultures differed from vehicle or medium controls (Tables 5, 6, 10, and 11, also appended here).

*No explanation or definition was provided for this term.

In both trials, the positive control (AAF) responded as expected, with highly significant increased net silver grain counts 14 and 23 times solvent values.

The author concluded that the test substance gave no indication of inducing DNA damage in rat hepatocytes (as measured by the induction of UDS).

F. TB Evaluation - UNACCEPTABLE. Although apparently conducted with adequate controls under accepted test guidelines, no indication was given in the entire report that the HDT, 170 ug/mL represented a cytotoxic or solubility limit for the test substance, as evident by the preliminary cytotoxicity testing reported (Tables 1 and 2).

ATTACHMENTS (Data Tables)

ATTACHMENT I

Data Tables

SIMAZINE

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