FYR-7449

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

AUG 24 1989

007449

MEMORANDUM

OFFICE OF
PESTICIDES AND TOXIC SUBSTANCES

SUBJECT: Simazine, review and/or reevaluation of data evaluation reports for SRR.

TO: James Yowell
Herbicide Branch
Registration Division (H7505)

FROM: Henry W. Spencer, Ph.D. 7/27/89
Section 2 , Toxicology Branch I (IRS)
Health Effects Division (H7509C)

THRU: Marion P. Copley, D.V.M., Section Head 8/17/89
Section 2, Toxicology Branch I (IRS) KA 8/22/89
Health Effects Division (H7509C)

Tox. Chem. No. 740
Project No. NA
Record No. NA

The following studies were either reevaluated or initially reviewed for the SRR on Simazine.

1. Acute oral LD 50 - rats, MRID 00148897
Study # 12218
Classification Core- supplementary

Classification is due to the lack of the purity designation in the report which must be submitted.

2. Acute Dermal Toxicity -rabbits, MRID 00148898
Study # 12218
Classification Core - Supplementary

Classification is due to the lack of the purity designation in the report which must be submitted.

3. Acute Inhalation Toxicity - rats, MRID 00148899
Study # 12218
Classification Core- Supplementary

Classification is due to the lack of the purity designation in the report which must be submitted.

4. Primary Eye Irritation -rabbits- MRID 00148900
Study # 1221D
Classification Core- Supplementary
Classification is due to the lack of the purity designation in the study report which must be submitted.

5. Primary Dermal Irritation - rabbits- MRID 00148901
Study # 1221E
Classification Core - Supplementary

Classification is due to the lack of the purity designation in the study report which must be submitted.

6. Dermal Sensitization - guinea pig- MRID 00148902
Study # 1221F
Classification Core- Supplementary

Classification is due to the lack of the purity designation in the study report which must be submitted. Supporting information on the absorptive capabilities of "paraffin oil" in guinea pigs are required.

7. Chronic Toxicity/Oncogenicity - rat- MRID 40614405
Study # 2-011-09
Classification Core - Minimum for both segments.

NOEL = 10 ppm (0.5 mg/kg/day)
LEL = 100 ppm (5.3 mg/kg/day) in female rats.
The LEL was based on depression of body weight gains and depression of values for the hematology parameters, RBC, HGB, and HCT in female rats.
Mammary tumors were induced in female rats at doses of 100 ppm and 1000 ppm (63.1 mg/kg/day)
Simazine appears to induce the formation of liver tumors (hepatocellular adenomas/carcinomas) at the dose level of 1000 ppm (45.8 mg/kg/day) in male rats.

8. Chronic (one year) feeding -dog - MRID 40614402
Study # 87122
Classification Core- Minimum

NOEL =20 ppm or 0.76 mg/kg/day for females (LDT)
LEL =100 ppm lor 3.6 mg/kg/day for decreased body weight gain, and decreases in RBC, HGB, HCT and a nominal increase in platelet counts in females.
At 45 mg/kg/day in females decreases occurred in body weight gain and in RBC, HGB, and HCT. At 43 mg/kg day in males decrements in body weight gain, and variable but reversible decrements in RBC, HGB, and HCT, and increases in platelet counts occurred.

9. Chronic Toxicity/Oncogenicity feeding-mouse- MRID 40614404
Study # 842121
Classification Core- Guideline
Simazine was not oncogenic in CD-1 mice when fed in the diet at 0, 40, 1000, or 4000 ppm for 95 weeks. There was a decrease in mean body weight in both males and females in the mid and high-dose groups, and a decrease in food consumption in mid- and high-dose males and in mid-dose females. There were decreases in erythroid parameters which may have been related to weight loss. Other hematologic parameters were not affected. Clinical chemistry values and urinary parameters were normal in dosed groups. Organ-to-body weight ratios were increased in high-dose females for several organs; however, there were no histologic correlates and the changes were accompanied by decreased terminal body weights. There were no nonneoplastic changes related to dosing. The incidence of amyloidosis was high in all groups. The LOEL based on decreased weight gain was 1000 ppm and the NOEL was 40 ppm.

10. Teratology- rat- MRID 40614403
report ~~Study~~ # 83058
Classification Core- Supplementary

Developmental (Embryo/fetal) Toxicity:
NOEL = 30 mg/kg/day
LEL = 300 mg/kg/day and higher for increased head incompletely ossified, teeth not ossified, centra/ vertebrae unossified and/or (additional), rudimentary ribs, presphenoid not ossified, and sternebrae not ossified. No malformations were reported.
Maternal Toxicity:
NOEL = 30 mg/kg/day
LEL = 300 mg/kg/day and higher for decreased maternal body weight and body weight gain, food consuption, and efficiency of food utilization. A/D ratio = 1.
Noted: That additional information is required to elevate the core classification.
The additional information required includes, 1. particle size and distribution of the sizes suspended in the test vehicle., 2. What kind of preparation was carried out on the test material prior to suspension?, 3.Sample analysis of dosing suspensions must be submitted., 4. Purity of the test material must be submitted., 5. The source of the test animals must be submitted.

11. Three-Generation Reproduction Study - rat - MRID 00023365, 00080631
Study # None
Classification Core- Supplementary

Parental Toxicity: NOEL = less than 50 ppm in male and females due to reduced weight gain in $F1_b$ and F_2b generations. The weights were also significantly reduced by approximately 11 percent in the F_0 generation during their premating period when compared to the controls. There were several males in groups F_0, F_1, and F_2 of the study which appeared unable to produce young but they were not evaluated.
There was a suggestion that the treated F_3b pups examined histologically may have altered livers but too few animals were examined to be able to completely evaluate this effect. A reproductive NOEL therefore cannot be determined due to the lack of evaluation of apparently sterile males.
Note: The study is down-graded.

12. Mutagenicity- Salmonella/mammalian Microsome assay
MRID 40614406
Study # 87038
Classification Core- Acceptable

Simazine was noncytotoxic and nonmutagenic at the limit of solubility (250 micrograms/plate).

13. Mutagenicity- Unscheduled DNA repair- rat hepatocytes
MRID 40614408
Study # 830640
Classification Core- Unacceptable

Cultures were exposed to a maximum of 50 microgram / ml for only 5 hours and did not show an increase in net nuclear granules counted. The length of exposure was to short to assure adequate exposure of the test cells. No data were presented to show a solubility maximum.

14. Mutagenicity- In vitro cytogenetic study with human lymphocytes.
MRID 40614407
Study # 871099
Classification Core- Unacceptable

At doses up to 100 micrograms/ml and both nonactivated and S9 activation neither cytotoxicity or clastogenic effects were seen. No information on the solubility of Simazine in the test system was reported. In addition, cell harvest was at 48.5 hours post treatment and did not ensure that the first division metaphases were available for analysis of compound effects.

15. Dermal Absorption - rat- ^{14}C-Simazine
MRID 40614409

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Study # ABR- 88042
Classification Core- Acceptable

When doses of 1 and 5 mg/rat (0.1 and 0.5 mg/cm^2) and exposures of 2, 4, 10, and 24 hrs, actual mean dermal absorption was found to be less than 1 %. However, between 11 and 20% of the low dose and 31 and 41% of the high dose remained on the skin after soap and water wash. This quantity is potentially absorbable.

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Reviewed By: Henry Spencer, Ph.D. 2/23/89
Section II, Toxicology Branch I - IRS (TS-769C)
Secondary Reviewer: Marion P. Copley, D.V.M. 2/23/89
Section Head, Section II, Toxicology Branch I - IRS (TS-769C)

DATA EVALUATION REPORT

Study Type: Acute Oral Toxicity in Rats TOX Chem No.: 740

Accession No.: N/A MRID No.:00148897

Test Material: Simazine, Technical, 1181

Synonyms: Simanex

Study No.: 1221A

Sponsor: Makhteshim-Agan (America), Inc.
2 Park Avenue
New York, NY 10016

Testing Facility: Cosmopolitan Safety Evaluation (CSE), Inc.
P.O. Box 71
Lafayette, NJ 07848

Title of Report: Acute Oral Toxicity Study in Rats;
Laboratory Report #1221A.

Author: Gerald Rosenfeld

Report Issued: March 25, 1985

Conclusions:

This study is adequate to establish an LD_{50} at greater than 5 g/kg bwt and Toxicity Category IV.

Classification:

Core Supplementary due to lack of active ingredient purity statement.

Special Review Criteria (40 CFR 154.7):

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Materials:

1. Test Compound - Simanex, Technical (simazine), a white powder (1181).

2. Test Animals - Young adult albino rats (Sprague-Dawley derived).

Study Design:

Five animals in each sex were given the simazine as a single dose in corn oil at 5 g/kg bwt. Immediately following dosing, observations were made often for the first 5 hours and then 2X daily for 14 days. Food and water were provided ad libitum.

Toxicity signs were recorded. After 14 days, survivors were necropsied.

Results:

Signs of toxicity included reduced activity, chromorhinorrhea, chromodacryorrhea, perineal staining, and emaciation. Weight losses occurred for a period of 7 days.

Only gastrointestinal tract congestion was seen in rats that died on study. No other necropsy findings were reported.

Mortality:

One of the five males died at day 6 of the study. Two of five females also died on day 6 of the study. Therefore, the LD_{50} is greater than 5 g/kg bwt. The study is adequate to place the technical chemical for labeling purposes into Toxicity Category IV.

Although 3 of the 10 animals tested on study died at day 6 after dosing (late in the study), Toxicology Branch considers it of little value to pursue an exact LD_{50} above 5000 mg/kg particularly since all but two of the remaining animals appeared normal. One of each sex exhibited toxicity as chromodacryorrhea or chromorhinorrhea with emaciation. By day 10 these rats also were normal appearing.

Reviewed By: Henry Spencer, Ph.D. 2/23/89
Section II, Toxicology Branch I - IRS (TS-769C)
Secondary Reviewer: Marion P. Copley, D.V.M. 2/23/89
Section Head, Section II, Toxicology Branch I - IRS (TS-769C)

DATA EVALUATION REPORT

Study Type: Acute Dermal Toxicity - Rabbits TOX Chem No.: 740

Accession No.: N/A MRID No.: 00148898

Test Material: Simazine, Technical

Synonyms: Simanex

Study No.: 1221B

Sponsor: Makhteshim-Agan (America), Inc.
2 Park Avenue
New York, NY 10016

Testing Facility: Cosmopolitan Safety Evaluation (CSE), Inc.
P.O. Box 71
Lafayette, NJ 07848

Title of Report: Acute Dermal Toxicity Study in Rabbits, Laboratory Report #1221B.

Author: Gerald Rosenfeld

Report Issued: March 25, 1985

Conclusion:

This study is adequate as a "limit test" to indicate that simazine technical has an LD_{50} of greater than 2.0 g/kg from dermal toxicity. The Toxicity Category is III.

Classification:

Supplementary for lack of active ingredient purity statement. The study may be upgraded.

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Materials:

1. Test Compound - Simanex, Technical (simazine) 1181, a white powder - Purity was not submitted.

2. Test Animals - Young adult albino rabbits weighing from 2.5 to 3.5 kg. Five/sex were exposed.

Study Design:

Each of 5/sex were randomly assinged to cages. The day before treatment approximately 80 percent of the animal trunk was clipped with an Oster clipper, and 2.0 g/kg of the test material was applied uniformly as a moistened powder over about 10 percent of the body surface. The treated area was then covered by gauze and tape followed by plastic sheeting.

After treatment at 24 hours, the test areas were cleansed of the dose.

Readings were made at 24 hours, and at 3, 7, and 14 days using a modified Draize method.

Results:

There were no clinical signs of toxicity. However, local effects included both erythema and edema which subsided by day 7. No deaths occurred.

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Reviewed By: Henry Spencer, Ph.D. 2/23/89
Section II, Toxicology Branch I - IRS (TS-769C)
Secondary Reviewer: Marion P. Copley, D.V.M. 2/27/89
Section Head, Section II, Toxicology Branch I - IRS (TS-769C)

DATA EVALUATION REPORT

Study Type: Acute Inhalation Toxicity Study in Rats

TOX Chem No.: 740

Accession No.: N/A

MRID No.: 00148899

Test Material: Simazine, Technical

Synonyms: Simanex

Study No.: 1221C

Sponsor: Makhteshim-Agan (America), Inc.
2 Park Avenue
New York, NY 10016

Testing Facility: Cosmopolitan Safety Evaluation (CSE), Inc.
P.O. Box 71.
Lafayette, NJ 07848

Title of Report: Acute Inhalation Toxicity Study in Rats, Study #1221C.

Author: Gerald Rosenfeld

Report Issued: March 21, 1985

Conclusion:

The data are sufficient to establish that an LC_{50} for Simanex technical is greater than 1.71 mg/L for a 4-hour exposure study; 1.71 mg/L was considered to be the maximal amount which could be generated and maintained in the chamber breathing zone.

Classification:

Core-Supplementary. May be upgraded with submission of a purity statement. This is considered by Toxicology Branch as a limit test. Toxicity III

-010

Materials:

1. Test Compound - Simanex, Technical (Simazine) 1181, a white powder. Purity statement of the ai is absent from the report.

2. Test Animals - Young adult Sprague-Dawley-derived rats (5/sex) were used.

Study Design:

A test group of 10 animals (5/sex) and a control group of equal size were used in the test chambers. Test material was passed through a Wright dust generator and passed into the chamber at the rate of 10 L/minute. The exposure period was 4 hours long. Exposed rats were observed for an additional 14 days. Actual exposure was determined by gravimetric measurement.

Observations:

Mortality and pharmacotoxic signs were reported during the exposure and observation period. Necropsy was carried out on survivors.

Results:

The nominal exposure to the test animals was 14.7 mg/L. Actual concentrations by gravimetric analysis was 1.71 mg/L. Each analytical determination found that approximately 80 percent of the mass median aerodynamic diameter (MMAD) was 7 microns or smaller.

The MMAD was approximatey 1.1 microns over the 4-hour period; 35 to 43 percent of the cumulative particle mass was determined to range from 0.8 to 0.3 microns in diameter.

Clinical signs were negligible except for wetting of muzzle fur during the exposure. Some decrease in activity occurred in about 1/2 of each sex.

Body weight was lost until day 7 when normal weight gains were evident.

No abnormalities were noted upon necropsy.

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Reviewed By: Henry Spencer, Ph.D. 2/23/89
Section II, Toxicology Branch I - IRS (TS-769C)
Secondary Reviewer: Marion P. Copley, D.V.M. 2/23/89
Section Head, Section II, Toxicology Branch I - IRS (TS-769C)

DATA EVALUATION REPORT

Study Type: Primary Eye Irritation Study in Rabbits

TOX Chem No.: 740

Accession No.: N/A

MRID No.: 00148900

Test Material: Simazine, Technical

Synonyms: Simanex

Study No.: 1221D

Sponsor: Makhteshim-Agan (America), Inc.
2 Park Avenue
New York, NY 10016

Testing Facility: Cosmopolitan Safety Evaluation (CSE), Inc.
P.O. Box 71
Lafayette, NJ 07848

Title of Report: Primary Eye Irritation Study in Rabbits, Laboratory Report #1221D.

Author: Gerald Rosenfeld

Report Issued: March 25, 1985

Conclusion:

The study is adequate to indicate that technical simazine (Simanex) is very slightly irritating to the eyes of rabbits.

Toxicity Category IV

Classification:

Core-Supplementary. A statement of the purity of the active ingredient was missing from the report. The study classification may be upgraded with submission of the missing information.

Materials:

1. Test Compound - Simanex, Technical (Simazine) 1181, as a white powder was used as received.

2. Test Animals - Young adult white rabbits weighing 2 to 3.5 kg. The animals were acclimated for 5 days prior to assignment.

Study Design:

A 0.1 g amount of test material was placed in the lower lid of one eye. The other eye was the control.

Feed and water were provided ad libitum. Observations were made at 1, 24, 48, and 72 hours. Positive effects were reported each 3 to 4 days thereafter until the irritation had subsided or was considered to be a prolonged effect. Grading of effects was by a modified Draize schedule.

Results:

Conjunctivitis (redness) was noted only at 1 hour in all six of the test animals. The effects were minimal and completely reversed by 24 hours. The study lasted only 72 hours.

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Reviewed By: Henry Spencer, Ph.D. 2/23/89
Section II, Toxicology Branch I - IRS (TS-769C)
Secondary Reviewer: Marion P. Copley, D.V.M. 2/22/89
Section Head, Section II, Toxicology Branch I - IRS (TS-769C)

DATA EVALUATION REPORT

Study Type: Primary Dermal Irritation in Rabbits

TOX Chem No.: 740

Accession No.: N/A

MRID No.: 00148901

Test Material: Simanex (Technical), Simazine

Study No.: 1221E

Sponsor: Makhteshim-Agan (America), Inc.
2 Park Avenue
New York, NY 10016

Testing Facility: Cosmopolitan Safety Evaluation (CSE), Inc.
P.O. Box 71
Lafayette, NJ 07848

Title of Report: Primary Dermal Irritation Study in Rabbits, Laboratory Report #1221E.

Author: Gerald Rosenfeld

Report Issued: March 25, 1985

Conclusion:

The study is adequate to indicate that simazine powder in a corn oil paste is very slightly irritating to the skin. A PIS of 0.2 was calculated by the Draize method.

Toxicity Category IV

Classification:

Core-Supplementary. Purity of the active ingredient was missing from the report. The study may be upgraded with the submission of the missing information.

Materials:

1. Test Compound - Simanex, Technical (simazine) 1181, as a white powder, was mixed as 0.5 g in 0.4 mL of corn oil per animal.

2. Test Animals - Six young adult white rabbits (sex not specified) weighing from 2.0 to 3.5 kg.

Study Design:

Six animals were clipped the day before the treatment commenced. The 0.5 g of paste was placed under a 1-inch gauze and covered with adhesive and impervious sheeting for a period of 4 hours.

One hour after cleaning the skin, irritation readings were made. Additional readings were made at 24, 48, and 72 hours. Scoring of effects was by the Draize method.

Results:

All animals were affected by 45 minutes. Irritation noted as erythema never got higher than a grade of 1. Only two animals remained at grade 1 at 24 and 48 hours. Complete reversal had occurred by 72 hours. A PIS was calculated as 0.2.

The study indicated that Toxicity Category IV can be established.

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Reviewed By: Henry Spencer, Ph.D. 2/23/89
Section II, Toxicology Branch I - IRS (TS-769C)
Secondary Reviewer: Marion P. Copley, D.V.M. 2/23/89
Section Head, Section II, Toxicology Branch I - IRS (TS-769C)

DATA EVALUATION REPORT

Study Type: Guinea Pig Sensitization Study

TOX Chem No.: 740

Accession No.: N/A

MRID No.: 00148902

Test Material: Simanex Technical (Simazine), a white powder

Study No.: 1221F

Sponsor: Makhteshim-Agan (America), Inc.
2 Park Avenue
New York, NY 10016

Testing Facility: Cosmopolitan Safety Evaluation (CSE), Inc.
P.O. Box 71
Lafayette, NJ 07848

Title of Report: Guinea Pig Sensitization Study (Buehler), Laboratory Report No. 1221F.

Author: Gerald Rosenfeld

Report Issued: March 25, 1985

Conclusion:

The study is not adequate to indicate that the test material Simanex (technical) does not have significant dermal sensitizing potential without further information on the absorptive capabilities of paraffin oil in guinea pigs.

Classification:

Core-Supplementary. The study may be upgraded with the submission and review of the purity statement and additional information on the use of "paraffin oil" in the study.

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Materials:

1. Test Animals - Ten young adult albino male guinea pigs were used in each test dose and positive control group.

2. Test Materials:

   a. Simanex, Technical (simazine) 1181, as a white powder. Purity of the active ingredient was not submitted.

   b. Positive Controls - a 2.0 w/w concentration of p-phenylenediamine in saline.

Study Design:

An induction phase of the study required the shaving (clipping) of the back of the guinea pigs. Simazine (0.5 g) in paraffin oil was applied topically under an occlusive patch for 6 hours, weekly for 3 weeks. A 2-week rest period followed and then a challenge phase of the study was commenced.

The challenge phase used the same quantity of test or positive control material at both the induction site and a virgin site. Readings of irritation were scored for each animal at each site at 24 and 48 hours after challenge.

Both erythema and edema as well as diameter of the reaction were recorded.

Results:

The positive control animals all exhibited extensive increases in severity and size of reaction when challenged.

However, only two animals exposed to simazine exhibited inconsistent results in the induction phase. No irritation was noted following the challenge phase.

There is some question as to whether paraffin oil allows the dissolved simazine to enter the animal's system to any degree.

Further data on (comparative) absorptive rates of this solvent in comparison to others normally used are required.

Reviewed By: Y.M. Ioannou 10/28/88
Section II, Toxicology Branch I - IRS (TS-769C)
Secondary Reviewer: M. Copley
Section II, Toxicology Branch I - IRS (TS-769C)

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DATA EVALUATION REPORT

Study Type: Chronic Toxicity/Carcinogenicity (Rat) (83-5)

TOX Chem No.: 740
MRID No.: 406144-05

Test Material: Simazine Technical

Study No(s).: 2-011-09

Sponsor: Ciba-Geigy Corporation, Greensboro, NC

Testing Facility: Ciba-Geigy Corporation
Pharmaceuticals Division
Summit, NJ

Title of Report: Simazine-Technical: 104-Week Oral Chronic Toxicity and Carcinogenicity Study in Rats.

Author(s): C.C. McCormick, A.T. Arthur, J.D. Green

Report Issued: April 12, 1988

Conclusions:

The LEL for chronic toxicity of Simazine Technical in Sprague-Dawley rats was found to be 100 ppm (5.3 mg/kg/day) based on depression of body weight gains and depression of values for the hematology parameters, RBC, HGB and HCT in female rats. The NOEL was found to be 10 ppm (0.5 mg/kg/day).

Simazine Technical was found to be oncogenic in female rats, inducing mammary tumors at dose levels of 100 ppm (5.3 mg/kg/day) and 1000 ppm (63.1 mg/kg/day).

In male rats Simazine appears to induce the formation of liver tumors (hepatocellular adenomas/carcinomas) at the dose level of 1000 ppm (45.8 mg/kg/day).

Classification: Core-Minimum

Materials and Methods:

Male and female Sprague-Dawley rats [Crl:VAF/Plus™ CD® (SD)Br] obtained from Charles River, Kingston, NY, approximately 6 weeks old and weighing 126 to 189 g (males) or 101 to 167 g (females) were used throughout this study. Upon arrival all animals were examined for their health status and only healthy animals were included in the study. Ophthalmoscopic examinations were performed on all animals and necropsy and serologic determinations were performed on five males and five females, randomly selected. The rats were acclimated to laboratory conditions for approximately 3 weeks and after the first week of acclimation they were housed in individual cages, identified with Monel ear tags and provided with food (Ground Purina Certified Rodent Chow #5002) and tap water ad libitum. Animal cages were kept in a room where the temperature was maintained at 73 ± 5 °F, the relative humidity at 50 ± 20 percent, with a 12-hour light/dark cycle.

Study Design:

A total of 340 male and 340 female rats were used in this study. The rats were randomly divided into four major groups/sex and exposed to dietary concentrations of Simazine Technical as shown on the following page (abstracted from the original report).

For the preparation of the test diets, Simazine Technical (Batch FL 850614) with a purity of 96.9 percent (personal communication with Tom Parshley of Ciba-Geigy) was mixed with powdered Certified Purina Rodent Chow #5002, at intervals based on the stability of the test article admixtures at room temperature. This stability was reportedly at least 21 days for the low-dose (10 ppm) and at least 40 days for the mid- and high-dose admixtures. Test article concentrations in the diet were determined at study initiation and at approximately 4-week intervals thereafter for the first year, and at 8-week intervals for the second year on study. The homogeneity of Simazine in diet admixtures was determined twice (study week 1 and 63) during the study.

All animals were observed daily for clinical symptoms of toxicity and mortality. Body weights were recorded on weeks -3 and -2, weekly during weeks 1 through 13, biweekly during weeks 14 through 25 and monthly thereafter for the remainder of the study. Food consumption was determined weekly for weeks 1 through 13, biweekly for weeks 14 through 25 and monthly thereafter. Water consumption was measured on weeks 1, 2, 53 through 64, and 102 on study. All animals were palpated for masses at 4-week intervals for the first 9 months on study and at 2-week intervals thereafter.

Treatment Schedule:

The test article/feed admixtures were available ad libitum at concentrations of 10, 100 or 1000 ppm. The control group received untreated Certified Purina Rodent Chow #5002 ad libitum. Chronic phase animals consisted of 40 rats/sex in the control and high-dose groups and 30 rats/sex in the low- and intermediate-dose groups, while carcinogenicity phase animals consisted of 50 rats/sex/group. The test article/ feed admixtures were administered 7 days/week for a minimum of 104 consecutive weeks according to the following schedule:

Group	Phase	Number of Rats		Dietary Concentration (ppm)	Least Number of Dose Weeks
		Male	Female		
1	Chronic[a]	10	10	0	52
		10	10		52 + 52-wk recovery
		20	20		104
	Carcinogenicity[b]	50	50		104
2	Chronic[a]	10	10	10	52
		20	20		104
	Carcinogenicity[b]	50	50		104
3	Chronic[a]	10	10	100	52
		20	20		104
	Carcinogenicity[b]	50	50		104
4	Chronic[a]	10	10	1000	52
		10	10		52 + 52-wk recovery
		20	20		104
	Carcinogenicity[b]	50	50		104

[a] After approximately 52 weeks of treatment, 10 rats/sex/group from the chronic phase were sacrificed and an additional 10 rats/sex also from the chronic phase in the control and high-dose groups were maintained on untreated (control) diet for approximately 52 weeks at which time the remaining animals were sacrificed. After approximately 104 weeks of treatment, the remaining animals from the chronic phase were sacrificed.

[b] After approximately 104 weeks of treatment, the remaining animals from the carcinogenicity phase were sacrificed.

Ophthalmoscopic evaluations were carried out on weeks -2, 25, 52, 72 through 76, and 104 and for recovery animals on week 65 on study. Blood smears for animals sacrificed moribund during the study were evaluated for differential count and red cell morphology.

For hematology and clinical chemistry determinations blood was collected from the right orbital sinus of male and female rats lightly anesthetized with ether. For urinalysis, urine samples were collected during a 16-hour overnight period from nonfasted animals for volume determinations while freshly voided urine was used for determination of all other urinalysis parameters. Hematology, clinical chemistry and urinalysis determinations were carried out based on the following schedule (abstracted from the original report):

				No. Rats Used for Clinical Lab. Determinations[a]					
	No. Rats		Week of	Hematology[b]		Biochem.[b]		Urinalysis[c]	
Group	M	F	Sac.	M	F	M	F	M	F
Baseline[c]	20	20	-1	10	10	10	10	10	10
1	10	10	105-106	10	10	10	10	10	10
	20	20	105-106	10	10	10	10	10	10
2	20	20	105-106	10	10	10	10	10	10
3	20	20	105-106	10	10	10	10	10	10
4	10	10	105-106	10	10	10	10	10	10
	20	20	105-106	10	10	10	10	10	10

[a] Animals from the carcinogenicity phase were used for these determinations at the final sampling period in order to have 10/sex/group.

[b] Analyses were conducted predose (test week -1) on baseline animals, at weeks 25 and 26, 77 and 78, and 104 on animals assigned to the 104-weeks chronic phase, and weeks 52, 65 and 66, 78 and 104 on animals assigned to the recovery phase.

[c] Baseline animals included 10/sex for hematology 10/sex for biochemistry and urinalysis. These data have been maintained in the raw data file for the study.

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For hematology, clinical chemistry, and urinalysis the following CHECKED (X) paramaters were examined:

1. Hematology

X		X	
X	Hematocrit (HCT)*		Total plasma protein (TP)
X	Hemoglobin (HGB)*	X	Leukocyte differential count
X	Leukocyte count (WBC)*	X	Mean corpuscular HGB (MCH)
X	Erythrocyte count (RBC)*	X	Mean corpuscular HGB conc. (MCHC)
X	Platelet count*	X	Mean corpuscular volume (MCV)
		X	Reticulocytes

2. Clinical Chemistry

X	Electrolytes:	X	Other:
X	Calcium*	X	Albumin*
X	Chloride*	X	Blood creatinine*
	Magnesium*	X	Blood urea nitrogen*
X	Phosphorous*	X	Cholesterol*
X	Potassium*	X	Globulins
X	Sodium*	X	Glucose*
	Enzymes	X	Total Bilirubin*
X	Alkaline phosphatase	X	Total Protein*
	Cholinesterase		Triglycerides
X	Creatinine phosphokinase*	X	A/G ratio
X	Lactic acid dehydrogenase		
X	Serum alanine aminotransferase (also SGPT)*		
X	Serum aspartate aminotransferase (also SGOT)*		
X	Gamma GT		

3. Urinalysis

X		X	
	Appearance*	X	Glucose*
X	Volume*	X	Ketones*
X	Specific gravity*	X	Bilirubin*
X	pH	X	Blood*
X	Sediment (microscopic)*		Nitrate
X	Protein*	X	Urobilinogen

Sacrifice and Pathology - All animals that died and that were sacrificed on schedule were subject to gross pathological examination and the CHECKED (X) tissues were collected for histological examination. The (XX) organs in addition were weighed.

*Recommended by Subdivision F (October 1982) Guidelines for chronic studies.

X		X		X	
	Digestive system		Cardiovasc./Hemat.		Neurologic
X	Tongue	X	Aorta*	XX	Brain*
X	Salivary glands*	XX	Heart*	X	Periph. nerve*
X	Esophagus*	X	Bone marrow*	X	Spinal cord (3 level)
X	Stomach*	X	Lymph nodes*		
X	Duodenum*	X	Spleen*	X	Pituitary*
X	Jejunum*	X	Thymus*	X	Eyes (optic n.)*
X	Ileum*		Urogenital		Glandular
X	Cecum*	XX	Kidneys*	XX	Adrenals*
X	Colon*	X	Urinary bladder*		Lacrimal gland
X	Rectum*	XX	Testes*	X	Mammary gland*
XX	Liver*	XX	Epididymides	X	Parathyroids*
	Gallbladder*	X	Prostate	X	Thyroids*
X	Pancreas*	X	Seminal vesicle		Other
	Respiratory	XX	Ovaries	X	Bone*
X	Trachea*	X	Uterus	X	Skeletal muscle*
X	Lung*			X	Skin
				X	All gross lesions and masses

*Recommended by Subdivision F (October 1982) Guidelines for chronic studies.

Histopathological examinations were conducted on all gross lesions involving tissue masses. In addition, formalin-fixed pituitary tissue was processed so that, if needed, sections could be stained using immunocytochemical staining procedures for the identification of prolactin.

Statistical Evaluation:

(Abstracted from the original report - see Appendix A)

Results:

Chemical analyses of feed admixtures established that a) Simazine was stable in the diet (at room temperature) for at least 21 days for the low-dose (10 ppm) and for at least 42 days for the mid- and high-dose levels. The authors did not give any justification as to why low-dose admixtures were tested for stability for only 21 days); b) Simazine concentrations in the diet were in close agreement with the target concentrations of 10, 100, and 1000 ppm; and c) Simazine homogeneity in diet admixtures was at an acceptable level as evidenced by the almost identical values obtained from samples within the same dose level.

Clinical Signs - Although a great variety of clinical signs were observed throughout the study, the incidence and/or frequency of these signs was for the most part comparable between the Simazine-treated and the control groups. Clinical signs that were of higher

incidence in the high-dose groups compared to controls included: Tissue mass in females (12 versus 38 for control and HDT, respectively); swollen appendages in males (7 versus 17 for control and HDT, respectively); alopecia/ general hairloss in females (2 versus 5 for control and HDT, respectively).

Mortality - As illustrated below, the mortality rate for the interim sacrifice and terminal sacrifice (main study) groups was very low during weeks 0 through 52 on study. However, a high rate of mortality was reported for the main study between weeks 53 and 106 (terminal sacrifice). For males the survival rate in the HDT was significantly higher than the control group (39 versus 60% for the control and HDT, respectively); on the contrary, in females the survival rate was in general lower than the males for all groups; the survival rate for the HDT females (20%) was much lower than the controls (34%).

Study	Week of Study	Sex	Mortality Ratio 0 ppm	10 ppm	100 ppm	1000 ppm
Interim	0-52	M	0/10 (0)[1]	1/10 (10)	0/10 (0)	0/10 (0)
		F	0/10 (0)	0/10 (0)	1/10 (10)	1/10 (10)
Main	0-52	M	2/70 (3)	0/70 (0)	0/70 (0)	0/10 (0)
		F	0/70 (0)	2/70 (10)	7/70 (10)	4/70 (6)
	53-106	M	41/68 (60)	46/70 (66)	39/70 (56)	28/70 (40)
		F	46/70 (66)	45/68 (66)	46/63 (73)	52/66 (79)
	0-106	M	43/70 (61)	46/70 (66)	39/70 (56)	28/70 (40)
		F	46/70 (66)	47/70 (67)	53/70 (76)	56/70 (80)

[1] Number in parentheses denotes percent mortality.

Palpable Masses - The incidence of palpable masses (confirmed at necropsy) was significantly higher in females of the HDT (1000 ppm) compared to the controls. For the controls 37/90 (41%) animals had palpable masses while for the high-dose group 60/80 (75%) animals had palpable masses. For the low- and mid-dose groups palpable masses were of approximately the same incidence as the controls. In males the incidence of palpable masses was comparable in all groups.

Ophthalmological Examinations - None of the ocular effects observed could be attributed to the test article since the incidence and frequency of these effects were comparable between treated and control groups.

Body Weight - Data presented here indicate that mean body weights for male and female rats of the HDT (1000 ppm) were statistically significantly lower than the control group beginning on day 7 on study and continuing to study termination (day 728). Table 1.

For female rats of the mid-dose group (100 ppm) statistically significantly lower mean body weights as compared to controls were observed at different time intervals throughout the study and at study termination. Mean body weight gains were also statistically significantly lower in male and female rats of the high-dose groups as compared to controls throughout the study. For male and female animals of the mid-dose groups (100 ppm) statistically significantly lower body weight gains were seen occasionally at different time intervals but not at study termination.

Food Consumption - A statistically significant reduction in food consumption was observed in male rats of the HDT (1000 ppm) beginning at day 7 (first time point measured) and continuing until day 700 on study (4 weeks before sacrifice), Table 2. Statistically significant depression of food intake was also reported for female rats of the HDT on days 7 through 560 on study, but not during the final 6 months on study (Table 2). The reduced food consumption in males and females of the HDT correlated with the lower body weight and body weight gains in the same groups throughout the study. In rats of the low- and mid-dose groups (males and females) change in food consumption was seen only rarely during the study.

Based on the food consumption and the animal body weight (at mid-period) the authors calculated the following mean daily dose intake in mg/kg for each treatment group for both sexes:

Sex	Group	Dietary Concentration (ppm)	mg/kg/day	Mean Daily Dose mg/kg/day	Range mg/kg/day
M	2	10	0.5	0.41	0.27 - 1.29
	3	100	5.0	4.17	2.75 - 13.12
	4	1000	50.0	45.77	37.48 - 112.40
F	2	10	0.5	0.52	0.30 - 1.36
	3	100	5.0	5.34	3.27 - 14.50
	4	1000	50.0	63.10	50.04 - 125.24

These results indicate that females were receiving mean daily doses, on a mg/kg basis, between 27 and 38 percent higher than the corresponding male dose groups. The range for mean daily doses was for the most part comparable between the two sexes.

Table 1
Mean Body Weights and Percent Body Weight Gains at Selected Time Intervals

	Sex	Day on Study	Dose (ppm) 0	10	100	1000
Mean body weight (g)	M	0	160.4 (1.3)[1]	161.3 (1.3)	160.4 (1.3)	158.8 (1.3)
		7	206.9 (1.6)	207.3 (1.9)	204.9 (1.6)	188.4** (1.5)
		98	542.9 (5.7)	538.3 (5.8)	529.6 (4.7)	434.7** (4.1)
		364	757.5 (10.7)	774.6 (9.9)	731.2 (9.0)	573.7** (7.0)
		532	795.5 (16.7)	835.1 (14.6)	782.4 (11.5)	592.1** (8.4)
		728	744.8 (29.3)	785.2 (31.9)	744.2 (18.2)	582.5** (10.5)
Mean body weight gain (%)	M	7	29.0 (0.3)	28.6 (0.9)	27.8* (0.3)	18.7** (0.3)
		98	239.1 (3.2)	234.3 (3.3)	231.1 (2.9)	174.2** (2.2)
		364	374.0 (6.4)	381.2 (5.7)	357.3 (5.7)	261.7** (3.9)
		532	399.4 (10.3)	417.6 (9.0)	392.1 (8.0)	277.3** (5.1)
		728	372.8 (19.8)	389.9 (20.8)	368.5 (13.0)	270.6** (6.2)
Body Weight Gain Change Compared to Controls (%)	M	7	-	-1.4	-4.1	-35.5
		98	-	-2.0	-3.3	-27.1
		364	-	+1.9	-4.5	-30.0
		532	-	+4.6	-1.3	-30.6
		728	-	+4.6	-1.2	-27.4

[1] Numbers in parentheses denote standard error.

* Statistically significantly different from controls; $p < 0.05$ and $p < 0.01$, respectively.

Table 1 (cont'd)

	Sex	Day on Study	Dose (ppm) 0	10	100	1000
Mean body weight (g)	F	0	133.6 (1.1)[1]	135.4 (1.2)	126.0 (1.8)	131.1 (1.0)
		7	156.8 (1.2)	157.2 (1.4)	150.1** (1.7)	143.7** (1.1)
		98	303.6 (3.1)	298.2 (3.7)	295.4 (4.2)	239.6** (2.3)
		364	451.0 (7.4)	451.7 (7.9)	424.7* (9.2)	321.3** (4.1)
		532	524.3 (13.0)	502.6 (12.1)	497.5 (14.2)	362.2** (9.2)
		728	570.2 (26.3)	543.3 (22.2)	473.0* (31.4)	440.2** (24.4)
Mean body weight gain (%)	F	7	17.4 (0.4)	16.2 (0.4)	19.6* (0.7)	9.6** (0.4)
		98	127.6 (1.9)	120.5* (1.9)	135.7* (2.8)	82.7** (1.2)
		364	237.9 (5.0)	233.8 (4.8)	240.0 (6.0)	146.1** (2.7)
		532	296.0 (10.2)	274.1 (8.9)	307.3 (11.3)	178.6** (6.7)
		728	331.3 (18.7)	314.0 (19.4)	301.3 (27.8)	238.2* (18.1)
Body Weight Gain Change Compared to Controls (%)	F	7	-	-6.9	-12.6	-44.8
		98	-	-5.6	+ 6.3	-35.2
		364	-	-1.7	+ 0.9	-38.6
		532	-	-7.4	+ 3.8	-39.7
		728	-	-5.2	- 9.1	-28.1

[1]Numbers in paretheses denote standard error.
*,**Statistically significantly different from controls; $p < 0.05$ and $p < 0.01$, respectively.

Table 2
Mean Food Consumption at Selected Time Intervals

Day on Study	Food Consumption (Grams/Week) Dose (ppm) Males 0	10	100	1000	Females 0	10	100	1000
7	141.5 (1.2)[1]	142.6 (2.1)	143.8 (1.3)	124.4** (1.3)	114.3 (1.4)	119.5* (1.3)	120.1* (2.1)	103.3** (1.7)
98	182.9 (2.1)	173.9** (2.3)	188.6 (2.2)	154.4** (2.0)	132.8 (1.7)	133.6 (1.9)	141.5** (2.1)	102.2** (1.7)
364	177.1 (2.5)	180.8 (2.7)	174.3 (2.5)	160.2** (1.6)	145.4 (2.5)	156.7* (2.6)	142.2 (2.4)	107.3* (1.6)
532	198.9 (3.3)	178.5 (4.4)	184.5 (3.2)	164.2** (2.7)	149.4 (3.0)	136.1 (4.3)	146.3 (4.9)	102.4** (4.2)
728	158.7 (5.9)	148.2 (7.8)	146.9 (6.0)	155.0 (5.1)	126.9 (6.2)	116.3 (7.1)	120.0 (10.5)	101.2 (4.1)

[1] Numbers in parentheses denote standard error.
*,**Statistically significantly different from controls; $p < 0.05$ and $p < 0.01$, respectively.

Water Consumption - Some differences in water consumption were seen between the treated and the control groups. These differences are not, however, considered toxicologically important due to their random ocurrence and the lack of a dose-response.

Hematology - As shown in Table 3, a number of hematology parameters appeared to be affected by Simazine treatment. This apparent treatment-related effect was pronounced mainly in the high-dose group females (1000 ppm) at most time points of sampling. Statistically significant changes between the control and high-dose group values were seen in females in the following parameters: Red blood cell (RBC) count-depressed at all time points; hemoglobin (HGB)-depressed on days 361, 537, and 725 on study; hematocrit (HCT)-depressed on days 361, 537, and 725 of sampling; mean corpuscular hemoglobin (MCHB)elevated on days 361, 537, and 725 of sampling; mean corpuscular hemoglobin concentration (MCHC)-elevated on day 174 of sampling; white blood cell count (WBC)-elevated on days 174, 361, 537, and 725 of sampling; neutrophils (percent)-elevated on day 361 of sampling; and lymphocytes-depressed on day 361 of sampling. Changes in these parameters, although only occasionally statistically significant, were also observed in the mid-dose group females (Table 3). Comparable changes between the control and the high-dose group were also seen in females of the recovery group.

In males, the MCHC was statistically significantly higher in the HDT compared to the control group on day 361 of sampling (with an apparent dose-related trend); the leukocyte count was statistically significantly lower than controls in the mid- and high-dose groups on day 537 of sampling. Other changes seen were not considered treatment-related. In males of the recovery group hematology parameter values were comparable for the most part between the HDT and the control groups. Statistically significant lower values were seen on day 537 for mean corpuscular volume (MCV) and on days 537 and 725 for MCHB.

Clinical Chemistry - A number of clinical chemistry parameters were found to be statistically significantly different between treated and control groups at different time intervals in both sexes. However, it appears that the only changes on clinical chemistry parameters that could possibly be attributed to Simazine treatment were the depression of glucose levels in female rats at all time points of sampling (Table 4). Glucose depression was also seen with the recovery group females at all time points tested except on day 725.

Table 3
Effect of Simazine on Selected Hematology Parameters - Female Rats

Parameter	Day of Test	Dose (ppm) Main Study 0	10	100	1000	Recovery Group 0	1000
RBC (x10 E6/Cmm)	174	7.1 (0.2)[1]	6.7 (0.2)	7.0 (0.3)	6.8 (0.1)		
	361	6.3 (0.1)	6.2 (0.1)	6.1 (0.1)	5.4** (0.2)	6.4 (0.1)	5.8* (0.2)
	537	6.9 (0.1)	6.8 (0.1)	6.8 (0.2)	5.8** (0.2)		
	725	6.4 (0.3)	6.6 (0.1)	5.6 (0.3)	5.0** (0.3)		
HGB (gm/dL)	361	14.1 (0.2)	14.2 (0.1)	14.2 (0.2)	12.7** (0.4)	14.3 (0.1)	13.3** (0.3)
	537	14.8 (0.2)	14.6 (0.2)	14.7 (0.3)	13.2** (0.2)	14.8 (0.2)	13.8* (0.3)
	725	14.5 (0.5)	14.5 (0.2)	12.7* (0.5)	12.3** (0.5)		
HCT (%)	361	41.4 (0.6)	41.2 (0.5)	41.5 (0.9)	36.1** (1.3)	42.6 (0.5)	38.2** (0.9)
	537	43.5 (0.5)	42.6 (0.5)	42.8 (1.5)	37.9** (0.9)	44.0 (0.6)	41.2* (0.9)
	725	41.4 (1.4)	41.2 (0.7)	36.4* (1.4)	34.3** (1.5)		
MCHB (micro gm)	361	22.3 (0.3)	22.7 (0.3)	23.3 (0.3)	23.7* (0.4)		
	537	21.3 (0.2)	22.6 (0.4)	21.8 (0.2)	22.8** (0.4)		
	725	22.6 (0.5)	22.0 (0.3)	22.8 (0.5)	24.6* (0.5)		
MCHC (%)	174	34.4 (0.2)	34.0 (0.3)	34.0 (0.3)	35.5* (0.3)		
WBC (x10 E3/Cmm)	361	6.3 (0.5)	6.6 (0.5)	8.2 (1.0)	8.6* (0.6)	6.7 (0.5)	7.8 (0.8)
	725	7.8 (1.1)	7.4 (0.7)	10.2 (1.3)	14.0** (1.7)		

[1]Numbers in parentheses denote standard error.
*,**Statistically significantly different from controls; $p < 0.05$ and $p < 0.01$, respectively.

Table 3 (cont'd)
Effect of Simazine on Selected Hematology Parameters - Female Rats

Parameter	Day of Test	Dose (ppm)					
		Main Study				Recovery Group	
		0	10	100	1000	0	1000
Platelet (x10E3/Cmm)	174	865.2 (55.2)[1]	1003.8 (53.5)	947.8 (43.5)	1140.4** (40.1)		
	361	871.7 (41.6)	888.2 (35.3)	945.4 (60.8)	1062.0* (32.0)		
	537	880.0 (49.0)	970.0 (37.8)	1014.9 (56.2)	1212.3** (44.5)		
	725	980.4 (64.2)	952.9 (45.0)	1224.9* (85.7)	1189.0 (46.1)		
Neutrophils (%)	361	16.3 (1.5)	21.1 (2.8)	22.2 (3.2)	33.6** (3.7)		
Lymphocytes (%)	361	78.7 (1.6)	72.9 (2.9)	69.5 (3.7)	61.9** (3.4)		

[1]Numbers in parentheses denote standard error.
*,**Statistically significantly different from controls; $p < 0.05$ and $p < 0.01$, respectively.

Also, alkaline phosphatase activity was elevated at all time points measured reaching statistical significance on days 361 and 455 of sampling, in females of the recovery group (note: for the recovery group only parameters of the control and high-dose group were measured). For the same group (recovery group, females) the activities of SGOT and SGPT were also depressed slightly throughout the study.

Urinalysis - Most of the urinalysis parameters measured were found to be comparable in the control and treated groups in both sexes. Statistically significantly higher urine volume was obtained on day 358 of analysis in the females of the HDT, and the males and females of the recovery group. Urine specific gravity was statistically significantly decreased on days 358 and 454 of analysis in females of the recovery group.

Organ Weights

a. Absolute Organ Weights - A statistically significant decrease in absolute organ weight was observed as follows: Brain, high dose males at the 52-week sacrifice; heart, high dose males at the terminal sacrifice; and liver, high dose females at the 52-week sacrifice (Table 5).

Table 4
Effect of Simazine on Selected Clinical Chemistry Parameters

Parameter	Day of Test	Dose (ppm) Males 0	Males 10	Males 100	Males 1000	Females 0	Females 10	Females 100	Females 1000
Glucose (mg/dL)	174					176.4 (4.4)	179.3 (6.4)	176.7 (9.1)	144.8** (4.7)[1]
	361					143.9 (4.0)	143.8 (4.8)	140.4 (5.0)	125.0* (4.0)
	537					148.0 (9.0)	147.9 (4.2)	134.6 (6.5)	127.1 (4.2)
	725	141.4 (8.1)	100.2** (9.7)	130.4 (6.3)	157.2 (5.6)	143.3 (7.4)	131.7 (7.0)	114.5* (10.3)	118.5 (4.2)
Cholesterol (mg/dL)	537					108.7 (8.7)	140.1 (14.6)	154.9* (10.5)	135.2 (7.7)
Total Bilir. (mg/dL)	361	0.38 (0.03)	0.34 (0.03)	0.25** (0.02)	0.25** (0.02)	0.46 (0.07)	0.51 (0.11)	0.35 (0.03)	0.22** (0.02)
	537	0.44 (0.08)	0.41 (0.05)	0.32 (0.05)	0.30 (0.02)	0.48 (0.11)	0.36 (0.08)	0.21* (0.02)	0.26 (0.04)
Albumin (gm/dL)	174	3.5 (0.04)	3.5 (0.04)	3.5 (0.05)	3.6* (0.04)				
	361	3.6 (0.09)	3.6 (0.07)	3.8 (0.07)	3.9* (0.04)				
Globulin (gm/dL)	174					2.4 (0.1)	2.4 (0.1)	2.5 (0.1)	2.7* (0.1)
	361	3.0 (0.09)	3.0 (0.11)	2.7* (0.10)	2.9 (0.07)				
Album./Globul.	174					1.9	1.8	1.8	1.7*
	725					1.4	1.4	1.4	1.3
Calcium (mg/dL)	361	10.21 (0.09)	9.99 (0.08)	9.90** 0.03)	9.95* (0.05)				
Sodium (meq/L)	725					142.5 (1.0)	144.1 (0.5)	145.1 (0.8)	145.7* (0.5)

[1]Numbers in parentheses denote standard error.
*,**Statistically significantly different from controls; $p < 0.05$ and $p < 0.01$, respectively.

b. Relative Organ Weights - In male rats of the high-dose group the relative weight for brain, liver and testes was statistically significantly higher than controls at 52 weeks and 104 weeks of sacrifice. In females, the relative weight of brain, heart, adrenal, kidney, liver, and ovaries at the interim sacrifice (52 weeks) was statistically significantly higher than controls in the high-dose group. For kidneys, statistical significance was also seen with the mid-dose group. Additionally, the relative weight of heart, kidney, and liver was statistically significantly higher than controls in the high-dose group at terminal sacrifice (104 weeks) (Table 5).

c. Organ-to-Brain Weight Ratios - Statistically significantly lower organ-to-brain weight ratios were observed for the heart of the high dose group males at the 104-week sacrifice and for the liver of the high-dose group females at the 52-week sacrifice (Table 5).

Table 5
Effect of Simazine on Organ Weights

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Organ	Dose (ppm) 0		10		100		1000	
	52 Weeks[1]	104 Weeks	52 Weeks	104 Weeks	52 Weeks	104 Weeks	52 Weeks	104 Weeks
Males								
Brain - Absolute (g)	2.29 (0.05)[2]		2.31 (0.03)		2.30 (0.03)		2.16* (0.03)	
- % of Bodyweight	0.32 (0.01)	0.35 (0.02)	0.30 (0.01)	0.33 (0.01)	0.31 (0.01)	0.34 (0.01)	0.37* (0.02)	0.42* (0.01)
Heart - Absolute (g)		2.15 (0.07)		2.15 (0.08)		2.08 (0.07)		1.82** (0.03)
- % of Brain		93.04 (3.02)		91.49 (3.44)		87.45 (2.55)		81.18** (1.73)
Liver - % of Bodyweight	3.11 (0.14)	2.56 (0.13)	2.95 (0.08)	2.41 (0.12)	3.00 (0.07)	2.53 (0.10)	3.50* (0.11)	3.07** (0.08)
Testes - % of Bodyweight	0.74 (0.03)	0.67 (0.05)	0.64 (0.03)	0.61 (0.04)	0.66 (0.04)	0.64 (0.03)	0.86* (0.04)	0.86** (0.03)
Females								
Brain - % of Bodyweight	0.42 (0.02)		0.43 (0.03)		0.50 (0.02)		0.64** (0.02)	
Heart - % of Bodyweight	0.24 (0.01)	0.30 (0.02)	0.25 (0.01)	0.30 (0.02)	0.27 (0.01)	0.32 (0.01)	0.33** (0.01)	0.37* (0.02)
Adrenal - % of Bodyweight	0.015 (0.001)		0.016 (0.001)		0.015 (0.001)		0.022** (0.001)	
Kidney - % of Bodyweight	0.54 (0.02)	0.65 (0.05)	0.58 (0.03)	0.65 (0.04)	0.63* (0.02)	0.68 (0.04)	0.77** (0.02)	0.85** (0.06)
Liver - Absolute (g)	15.10 (0.84)		15.94 (0.73)		13.43 (0.76)		12.42* (0.72)	
- % of Brain	741.4 (34.8)		759.4 (40.7)		649.1 (32.7)		606.2* (36.4)	
- % of Bodyweight	3.08 (0.10)	2.32 (0.11)	3.17 (0.13)	2.35 (0.11)	3.18 (0.12)	2.54 (0.12)	3.81** (0.11)	3.33** (0.07)
Ovary - % of Bodyweight	0.021 (0.001)		0.022 (0.002)		0.022 (0.003)		0.030* (0.004)	

[1] Interim sacrifice.
[2] Numbers in parentheses denote standard error.
*,** Statistically significantly different from controls; $p < 0.05$ and $p < 0.01$, respectively.

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Gross Pathology - Gross pathology, performed on all animals that died during the study, sacrificed at moribund condition or sacrificed at the scheduled study period (52 weeks or 104 weeks on study), revealed that the incidence of macroscopic lesions in the simazine treated groups was not statistically significantly different from that of the control groups in either sex. Numerical differences in the incidence of gross lesions were seen in some instances, especially in the high-dose groups, and are reported in Table 6 for the record.

Histopathological Lesions - Histopathological examination revealed numerous nonneoplastic and neoplastic lesions in many tissues of male and female rats.

Male Rats - Nonneoplastic Lesions - As shown in Table 7, the incidence of a number of noneoplastic lesions in male rats was comparable between the controls and the low- and mid-dose groups tested, but slightly higher than controls in the high-dose group.

Neoplastic Lesions - Although not statistically significant, the incidence of several neoplastic lesions in male rats was numerically higher than controls mainly in the high dose group. Table 7 shows that these neoplastic lesions involved: adrenal-cortical adenoma; kidney-adenoma and carcinoma; liver-adenoma and carcinoma; and thyroid-C-cell adenoma and carcinoma.

Female Rats - Nonneoplastic Lesions - The incidence of several nonneoplastic lesions in female rats was statistically significantly higher in the high dose group compared to controls as follows: mammary gland-cystic glandular hyperplasia; liver-hematopoiesis; and spleen-hematopoiesis (Table 8). Other nonneoplastic lesions were found to be only numerically higher than controls mainly in the high-dose group as shown in Table 8.

Neoplastic Lesions - The incidence of mammary gland carcinomas in female rats was found to be statistically significantly higher than controls in the mid- and high-dose groups as shown in Table 8. The incidence of mammary gland fibroadenomas was also statistically significantly higher than controls in the high-dose group. Mammary gland adenomas were only numerically higher than controls in the low- and high-dose groups. The incidence of pituitary adenomas was extremely high in all groups including controls (Table 8). Pituitary carcinomas were of higher incidence in the low- and high-dose groups compared to controls. Although the incidence of kidney tubular adenomas was only 2/70 in the high-dose group (and 0/70 in the other groups), because of its rarity in Sprague-Dawley rats this tumor appears in Table 8 for the record.

Table 6
Summary of Macroscopical Observations

Macroscopical Observation	Dose (ppm)							
	Males				Females			
	0	10	100	1000	0	10	100	1000
Main Study (104 Weeks)								
Kidney - distended	2/70[1]	2/70	0/70	3/70	2/70	3/70	2/70	9/70
Ovary - cyst					2/70	2/70	2/70	4/70
Pituitary - enlarged	23/70	26/70	29/70	20/70	52/70	48/70	47/70	62/70
Postappendage - tissue mass					5/70	1/70	2/70	12/70
Skin (chest and thorax) - tissue mass	1/70	1/70	3/70	7/70	14/70	18/70	9/70	40/70
Skin (inguinal) - tissue mass					23/70	22/70	22/70	37/70
Spleen - enlarged					2/70	2/70	1/70	9/70
Interim Sacrifice (52 Weeks)								
Pituitary - enlarged					1/10	3/10	3/10	4/10
Skin (inguinal) - tissue mass					0/10	0/10	1/10	4/10
Recovery Group (104 Weeks)								
Skin (chest and thorax) - tissue mass					1/10			5/10

[1]Number of rats with specified observation/total number of tissues examined.

Table 7
Summary of Histopathological Lesions - Male Rats

Histopathological Observation[1]	Dose (ppm) 0	10	100	1000
Neoplastic Lesions				
Adrenal - cortical adenoma	0/69[2]	0/70	1/69	2/69
Kidney - Adenoma	0/70	0/70	0/70	1/70
- Carcinoma (primary)	0/70	0/70	0/70	2/70
Liver - Hepatocellular adenoma	1/70	1/70	0/70	3/70
- Hepatocarcinoma	0/70	2/70	4/70	3/70
- Combined adenoma and/or carcinoma	1/70	3/70	4/70	6/70
Thyroid - C-cell adenoma	2/70	7/69	5/69	6/70
- C-cell carcinoma	2/70	1/69	1/69	3/70
- Combined adenoma and/or carcinoma	4/70	8/69	6/69	9/70
Pituitary - Adenoma	42/69	47/70	47/70	38/70
Nonneoplastic Lesions				
Adrenal - Cortical hypertrophy/ cystic degeneration	7/69	4/70	6/69	13/69
- Focal cortical hyperplasia	2/69	2/70	3/69	7/69
Liver - Hyperplasia	2/70	0/70	0/70	0/70
Pituitary - Hyperplasia	12/69	14/70	10/70	15/70
Skin - Chronic lymphocytic inflammation	1/70	0/68	1/69	5/70
Testes - Focal interstitial cell hyperplasia	6/70	2/70	8/70	11/70
Thyroid - Focal interstitial cell hyperplasia	7/70	3/69	5/69	9/70

[1]Main study only (interim sacrifice and recovery groups not included).
[2]Number of rats with specified observation/total number of tissues examined.

Table 3
Summary of Histopathological Lesions - Female Rats

Histopathological Observations[1]	Dose (ppm) 0	10	100	1000
Neoplastic Lesions				
Mammary - Adenoma	2/70[2]	4/70	1/70	5/70
- Carcinoma	14/70	13/70	19/70*	35/70***
- Fibroadenoma	22/70	27/70	19/70	40/70**
Pituitary - Adenoma	62/70	57/70	60/70	57/70
- Carcinoma	1/70	3/70	0/70	6/70
Kidney - Adenoma (tubular)	0/70	0/70	0/70	2/70
Nonneoplastic Lesions				
Mammary - Cystic glandular hyperplasia	51/70	50/70	53/70	65/70***
Pituitary - Hyperplasia	2/70	6/70	3/69	2/70
Kidney - Hydronephrosis	3/70	0/70	0/70	6/70
- Epithelial hyperplasia pelvic	0/70	0/70	0/70	3/70
Adrenal - Focal medul. hyperplasia	0/70	4/70	3/70	3/70
Liver - Hematopoiesis	0/70	1/70	1/70	5/70*
Spleen - Hematopoiesis	0/70	1/70	1/70	4/70*
Thyroid - Focal interstitial cell hyperplasia	0/70	2/70	2/70	4/70

[1] Main study only (interim sacrifice and recovery groups not included).
[2] Number of rats with specified observation/total number of tissues examined.
*, **, *** Indicates significance at $p < 0.05$, $p < 0.01$, and $p < 0.001$, respectively.

Discussion:

The present study has investigated the chronic toxicity and oncogenic potential of simazine in male and female Sprague-Dawley rats. The selection of the dose levels used in this study (10, 100 and 1000 ppm) was based on the results of a 90-day feeding study in rats whereby the dose levels of 2000 and 4000 ppm resulted in significant body weight depression (20-40% compared to controls) while the low dose of 200 ppm was established as the NOEL (personal communication with Mr. Tom Parshley of Ciba-Geigy).

Analytical data presented by the authors indicate that: simazine concentrations in the diet were approximately the same as target concentrations (of 10, 100, or 1000 ppm); the test article was homogeneously distributed in the diet (for all dose levels); and the test article was stable in the diet for at least 40 days for the mid- and high-dose levels and 21 days for the low-dose level.

The clinical signs were approximately of equal incidence between the control and the simazine-treated groups. The occasional higher incidence of some clinical signs that was seen with the HDT was not considered to be treatment-related due to the lack of dose-response and/or the fact that this higher incidence did not persist throughout the study. Female animals of the high-dose group had a higher incidence of palpable masses, reflecting the higher incidence of tumors found in this group, as compared to controls.

Mortality data presented here indicate that mortality rates in female rats were very high in all groups (control and treated) with the MDT and HDT resulting in slightly higher mortality than the control group. Mortality rates in male rats were reported to be slightly lower than controls for the MDT and HDT. Further statistical analysis of the mortality rates in both sexes (conducted by C.J. Nelson, Science Analysis and Coordination Branch, Health Effects Division) has shown that in female rats mortality was statistically significantly higher in the mid- and high-dose groups with a statistically significant increasing trend; in male rats, mortality was statistically significantly decreased in the HDT compared to the control group with a statistically significant decreasing trend as shown below.

	Mortality	
Dose	Male	Female
0	48/80** (60)	53/80** (66)
10	47/71 (66)	47/70 (67)
100	39/70 (56)	53/71** (75)
1000	28/70** (40)	57/71** (80)

( ) Denotes percent.
Significance of trend denoted at control.
Significance of pair-wise comparison with control denoted at dose; ** p < 0.01

These findings might suggest a sex-related difference in susceptibility to the test article possibly resulting from the higher incidence of life-threatening tumors in female than in male rats.

Mean body weights and mean body weight gains for male and female rats of the high-dose groups, were statistically significantly lower than controls throughout the study. Terminal mean body weights were 22 and 23 percent lower than controls in males and females of the HDT, respectively, while mean body weight gains (at study termination) were depressed by 27 and 28 percent in males and females, respectively. In females of the MDT there was a 17 percent decrease in mean body weights and 9 percent body weight gain decrement at study termination. No effect was seen in males of the MDT. However, according to the authors, female animals received 28-38 percent higher concentrations of the test article throughout the study. This finding might partly explain the higher toxicity observed in females of the MDT. The lower mean body weights and body weight gains correlated with the statistically significantly lower food consumption for male and female rats of the HDT compared to controls. These results suggest that the lower body weight gains could be attributed, at least to some extent to the lower food intake possibly due to the unpalatability of the test article in the diet. However, a closer look at food consumtion- grams of food consumed per kg body weight- indicated that food intake for male and female rats of the HDT was significantly higher than the other groups, ranging from 5.7 percent (at day 98) to 25 percent (at day 728) for males and from 17 percent (at day 98) to 55 percent (at day 728) for females, suggesting that food efficiency for animals of the HDT was very low compared to the other groups.

Hematology data indicate that treatment of female rats with Simazine at 1000 ppm results in anemic animals as indicated by the simultaneous statistically significant decrease in RBC, HGB, and HCT at different time points of sampling. We request, however, that the authors provide the Agency with the appropriate bone marrow determinations (Myeloid/Erythroid ratio) for further evaluation of this effect (see Appendix B). Other parameters that appeared to be affected by the high dose of the test article included the statistically significant increase in WBC, MCHC, MCHB, and platelets and neutrophils, indicating in general an abnormal state in these animals. No major changes in these parameters between the treated and control groups were reported in male rats.

From the clinical chemistry parameters measured only the changes seen in glucose values in the females of the HDT appeared to be treatment-related. The lower glucose values, however, might be the indirect result of depressed body weights in this group.

Although the absolute and/or relative weights of a number of organs were statistically significantly different between the control and treated groups, such differences do not appear to be

of major toxicological significance since changes in organ weights, in general, were not associated with concomitant clinical chemistry changes and/or changes in pathological lesions (macroscopic and/or microscopic) in the same organs which could explain these organ weight changes.

The following points can made concerning the oncogenic potential of simazine in male and female Sprague-Dawley rats:

1. Female Rats

a. Mammary Gland - In female rats of the main study there was a statistically significant increase in the incidence of mammary carcinomas in the mid- and high-dose groups compared to controls. A statistically significantly higher incidence of fibroadenomas was seen in the high-dose group. When the incidence of these lesions was calculated separately for female animals that died (or sacrificed moribund) or animals that survived to terminal sacrifice, the following incidence of mammary tumors was seen.

	Lesion	Dose (ppm) 0	10	100	1000
Early deaths (prior to terminal sacrifice)	Adenoma	2/46[1]	3/47	1/53	4/56
	Carcinoma	10/46	9/47	12/53	28/56**
	Fibroadenoma	14/46	17/47	11/53	28/56**
Scheduled sacrifice (104 weeks)	Adenoma	1/24	1/23	0/17	1/14
	Carcinoma	4/24	4/23	7/17	7/14*
	Fibroadenoma	8/24	10/23	8/17	12/14*
Combined incidence	Adenoma	3/70	4/70	1/70	5/70
	Carcinoma	14/70	13/70	19/70*	35/70**
	Fibroadenoma	22/70	27/70	19/70	40/70**

[1] Number of animals with specified observation/total number of tissues examined.

*, **, *** Indicates significance at $p < 0.05$, $p < 0.01$, and $p < 0.001$, respectively.

As the statistical analyses carried out by the authors for different tumors in male and female rats were determined to be inadequate, further statistical evaluation for the major tumors listed in Tables 7 and 8, was conducted by D.J. Nelson, Statistician Science Analysis and Coordination Branch, Health Effects Division. Data presented in all tables below are the combined tumor incidence from the 52-week interim sacrifice and the 104-week study. The incidence of mammary tumors in female rats is presented in the following table.

Simazine Sprague-Dawley Rat Study--Female Mammary Gland Tumor Rates+ and Peto Prevalence Test Results

Dose (ppm)	0.000	10.000	100.000	1000.000
Adenoma Fibroadenoma	23/39 (26)	20/78a (26)	11/71 (15)	21/75 (28)
	p = 0.0689	p = 0.302	p = 0.177	p = 0.103
Carcinoma	16/39 (18)	13/80 (16)	20/75b (27)	40/78 (51)
	p < 0.0001**	p = 0.4740	p = 0.0392*	p < 0.0001**
Adenoma Carcinoma	39/39 (44)	33/80 (41)	31/75 (41)	61/78 (78)
	p < 0.0001**	p = 0.4064	p = 0.2229	p < 0.0001**

a First adenoma observed at 48 weeks in dose 10 ppm and the first fibroadenoma observed at 52 weeks in dose 0, 10, and 1000 ppm.
b First carcinoma observed at 48 weeks in dose 100 ppm.
+ Number of tumor-bearing animals/Number of animals at risk (excluding animals that died before the observation of the first tumor or animals not examined).
( ) Percent
Note: Significance of trend denoted at Control. Significance of pair-wise comparison with control denoted at Dose level.
* denotes p < 0.05 and ** denotes p < 0.01

These results indicated that there was a statistically significant dose-related trend in mammary carcinomas and in combined adenomas and carcinomas. The incidence of mammary carcinomas was statistically significantly increased in the mid- and high-dose groups compared to controls; also the incidence of combined adenomas and carcinomas was significantly higher in the HDT compared to controls. Mammary carcinomas in the main study (104-week sacrifice) contributed, according to the authors, to the increased mortality in the high-dose group animals (1000 ppm). A higher incidence of mammary carcinomas was also seen in the recovery study (52 weeks of treatment with 1000 ppm followed by 52 weeks of recovery) (vs. 4/10, for the control and HDT, respectively).

In female rats the incidence of hyperplastic changes (cystic glandular hyperplasia) in the mammary gland was statistically significantly higher than controls

in the HDT. This finding corroborates the observed high incidence of tumors in the HDT. It is generally understood that the higher tumor incidence correlates directly with a higher incidence of hyperplastic changes.

c. Pituitary Gland - In female rats the incidence of pituitary (pars distalis) carcinoma was found to be higher than controls in the HDT. The authors reported that this incidence was statistically significant when the Peto life table method of analysis was used. The incidence of adenomas was found to be extremely high in all groups but the authors reported that the incidence in the mid- and high-dose groups was statistically significantly increased when Peto's method was used for analysis (when contribution to death be considered). Further statistical analysis of these tumors (total tumor analysis) indicated, as shown below, that the incidence of combined adenomas/carcinomas in the mid- and high-dose groups was statistically significantly higher than controls with a significant dose-related trend.

Simazine Sprague-Dawley Rat Study--Female Pituitary Gland Tumor Rates+, Fatal Tumor Analysis and Generalized K/W Test Results

Dose (ppm)	0.000	10.000	100.000	1000.000
Adenoma	73/89 (82.0)	57/81 (71.2)	63/77[a] (81.8)	61/79 (77.2)
	p = 0.0033**	p = 0.3944	p = 0.3206*	p = [illegible]
Carcinoma	1/73 (1.4)	3/61 (4.9)	0/61 (0.0)	4/61 (6.6)
	p = 0.0010**	p = 0.0351	p = 0.4545	p = 0.[illegible]
Adenoma Carcinoma	74/89 (83.1)	60/81 (74.1)	63/77 (81.8)	65/79 (82.3)
	p = 0.0005**	p = 0.1361	p = 0.3261*	p = 0.[illegible]

+ Number of tumor bearing animals/Number of animals at risk (excluding animals not examined).
Percent
a First adenoma observed at 85 weeks in dose 100 ppm
b First carcinoma observed at 72 weeks in dose 1000 ppm.
Note: Significance of trend denoted at Control. Significance of pair-wise comparison with control denoted at Dose level.
* denotes p ≤ 0.05 and ** denotes p ≤ 0.01

The authors reported that these tumors (adenomas and carcinomas) were considered to be fatal "by virtue of their size and compression of the mid-brain," and thus contributed to the decreased survivability of the mid- and high-dose group females. Although these tumors (adenomas/carcinomas) were of approximately the same numerical incidence in all groups (treated and control) examination of the Kaplan-Meier survival curves (constructed by C.J. Nelson, Statistician, SACB/HED) indicates that the onset of these tumors is 4 to 15 weeks earlier in the mid- and high-dose groups as compared to the control and low dose groups.

For further evaluation of these tumor data the authors are requested to provide the Agency with historical control data as shown in Appendix B. Furthermore, the authors should provide the Agency with the results of the immunocytochemical staining of the pituitary for identification of prolactin (see Appendix B).

c. Kidney - Based on Peto's time-adjusted trend analysis the incidence of kidney tubular adenoma in female rats of the high-dose group was statistically significantly higher than controls. Additional analysis of these data (see below) indicated that there was a statistically significant dose-related trend for the incidence of this tumor. This tumor is considered to be very rare with a spontaneous

Simazine Sprague-Dawley Rat Study--Female Kidney Tubule Tumor Rates, Cochran-Armitage Trend Test and Fisher's Exact Test Results

Dose (ppm)	0.000	10.000	100.000	1000.000
Adenoma	0/74 (0.0)	0/62 (0.0)	0/64 (0.0)	2/55[a] (3.6)
	p = 0.0042**	p = 1.0000	p = 1.0000	p = 0.1794

[a]First adenoma observed at 71 weeks in dose 1000 ppm. No carcinomas were noted.
-Number of tumor-bearing animals/Number of animals at risk (excluding animals that died before the observation of the first tumor or animals not examined).
Percent
Note: Significance of trend denoted at Control. Significance of pair-wise comparison with control denoted at Dose level.
* denotes p < 0.05 and ** denotes p < 0.01

incidence of 0 to 1 percent in this strain of rats, as compared to 3.6 percent incidence in the high-dose group in this study. This finding does not appear to be of major biological significance. The sponsor is however requested to provide the Agency with historical control data for this tumor, as shown in Appendix B.

2. Male Rats

a. Liver - In male rats the incidence of hepatocellular adenomas or carcinomas was very low in all treated and control groups (0-5%). As shown in the table below, the incidence of combined adenomas and carcinomas was statistically significantly higher in the high dose group compared to controls possibly suggesting oncogenic potential of simazine to male rats.

Simazine Sprague-Dawley Rat Study--Male Liver Tumor Rates+, Cochran-Armitage Trend Test and Fisher's Exact Test Results

Dose (ppm)	0.000	10.000	100.000	1000.000
Adenoma	1/88 (1.1)	2/79[a] (2.5)	0/80 (0.0)	3/80 (3.8)
	p = 0.0824	p = 0.4594	p = 0.5238	p = 0.2752
Carcinoma	0/88 (0.0)	2/79 (2.5)	4/80[b] (5.0)	3/80 (3.8)
	p = 0.2169	p = 0.2223	p = 0.0494*	p = 0.1058
Adenoma Carcinoma	1/88 (1.1)	4/79 (5.1)	4/80 (5.0)	6/80 (7.5)
	p = 0.0643	p = 0.1519	p = 0.1554	p = 0.0448*

[a]First adenoma observed at 52 weeks in dose 10 ppm.
[b]First carcinoma observed at 99 weeks in dose 100 ppm.
+Number of tumor-bearing animals/Number of animals at risk (excluding animals that died before 52 weeks or animals not examined).
Note: Significance of trend denoted at Control. Significance of pair-wise comparison with control denoted at Dose level.
* denotes $p < 0.05$ and ** denotes $p < 0.01$

The incidence of hyperplastic changes, however, was very low in the control (2/70) and nonexistent in the treated groups (0/70, Table 7).

b. Thyroid - Although the incidence of combined thyroid C-cell adenomas and carcinomas was numerically higher in all treated groups as compared to controls, as shown below there was no significant dose-related trend or statistical significance between treated and control groups. The incidence of hyperplastic changes was comparable to the incidence of tumors for each group.

Simazine Sprague-Dawley Rat Study--Male Thyroid C-cell Tumor Rates+, and Peto Prevalence Test Results

Dose (ppm)	0.000	10.000	100.000	1000.000
Adenoma	2/52 (4)	7/52[a] (13)	5/51 (10)	6/58 (10)
	p = 0.3355	p = 0.0606	p = 0.1082	p = 0.0871
Carcinoma	2/34 (6)	1/31 (3)	1/36 (3)	3/45[b] (7)
	p = 0.1762	p = 0.1082	p = 0.2881*	p = 0.4183
Adenoma Carcinoma	4/52 (8)	8/52 (15)	6/51 (12)	9/58 (16)
	p = 0.1924	p = 0.1965	p = 0.2261	p = 0.1505

[a]First adenoma observed at 89 weeks in dose 10 ppm.
[b]First carcinoma observed at 102 weeks in dose 1000 ppm.
+Number of tumor-bearing animals/Number of animals at risk (excluding animals that died before the observation of the first tumor or animals not examined).
() Percent
Note: Significance of trend denoted at Control. Significance of pair-wise comparison with control denoted at Dose level.
* denotes $p < 0.05$ and ** denotes $p < 0.01$

c. Kidney - As shown below a very low incidence of tubular adenomas and carcinomas was seen in male rats. A statistically significant dose-related trend was observed for the incidence of carcinomas as well as the incidence of combined adenomas and carcinomas. As in female rats, the very low incidence of this rare tumor in male rats does not appear to be of biological significance.

Simazine Sprague-Dawley Rat Study--Male Kidney Tubule Tumor Rates+ and Peto Prevalence Test Results

Dose (ppm)	0.000	10.000	100.000	1000.000
Adenoma	0/51 (0)	0/46 (0)	0/48 (0)	1/57[a] (2)
	p = 0.0543	p = 1.0000	p = 1.0000	p = 0.5278[b]
Carcinoma	1/66 (2)	0/62 (0)	0/64 (0)	2/65[c] (3)
	p = 0.0332*	p = 0.1660	p = 0.1821	p = 0.2091
Adenoma Carcinoma	1/66 (2)	0/62 (0)	0/64 (0)	3/65 (5)
	p = 0.0056**	p = 0.1410	p = 0.1721	p = 0.1087

[a]First adenoma observed at 92 weeks in dose 1000 ppm.
[b]The p values for adenomas were calculated using the Cochran-Armitage Trend Test and Fisher's Exact Test, since the Peto Prevalence method collapsed to one interval.
[c]First carcinoma observed at 78 weeks in dose 1000 ppm.
+Number of tumor-bearing animals/Number of animals at risk (excluding animals that died before the observation of the first tumor or animals not examined).
( ) Percent
Note: Significance of trend denoted at Control. Significance of pair-wise comparison with control denoted at Dose level.
* denotes $p < 0.05$ and ** denotes $p < 0.01$

Based on the aforementioned evaluation of the data we conclude that Simazine Technical is oncogenic in female Sprague-Dawley rats inducing the formation of mammary gland carcinomas. Simazine Technical also appears to increase the induction of liver tumors in male rats. We thus consider this chemical a candidate for Peer Review.

Conclusions:

The LEL for the chronic toxicity of Simazine Technical in Sprague-Dawley rats was found to be 100 ppm (5.3 mg/kg/day) for females (depression of body weight gains and depression of values for the hematology parameters, RBC, HGB and HCT). In males the LEL was found to be 1000 ppm (45.8 mg/kg/day) based on depression of body weight gains. The NOEL was 10 ppm (0.5 mg/kg/day) for females and 100 ppm (4.2 mg/kg/day) for males.

Simazine Technical was found to be oncogenic in female Sprague-Dawley rats inducing mammary tumors at dose levels of 100 ppm (5.3 mg/kg/day) and 1000 ppm (63.1 mg/kg/day).

In male rats Simazine appears to induce the formation of liver tumors at the dose level of 1000 ppm (45.8 mg/kg/day).

Classification: Core-Minimum

STATISTICAL EVALUATION:

Body Weight, Food and Water Consumption, Clinical Laboratory and Organ Weight Data: All numerical data that were generated in the course of the study were stored in the Beckman TOXSYS® data base in the IBM mainframe computer and maintained by Research Computing Services in the SEF. Individual animal data reports were generated by programs in the Beckman TOXSYS® system or programs developed by Research Computing Services. Statistical analyses were performed separately for each sex using the Statistical Analysis System (SAS) Version 5 and SUGI Supplemental Library, 1983 Edition on the IBM mainframe computer.

Tests for outliers and Bartlett's test for homogeneity of variances were performed to check deviations from the normal theory model. If the model assumptions were met, Dunnett's tests were performed to compare each of the treated groups versus the control. If significant model deviations were detected (either outliers were present or heterogenous variances were evident), supplemental analyses, including the use of appropriate data transformations, nonparametric tests or other multiple comparison procedures without assuming equal variances, were performed as needed. Descriptions of specific methods employed and additional references were added in the summary tables when supplemental analyses were performed. Nonparametric tests based on ranks were conducted on parameters that were known not to be normally distributed. A detailed description of the statistical methodology used in this study is presented in Section 6.

Pathology: All microscopic data were recorded by the pathologist or designee into the NO3 Pathology Data system in the Ardsley IBM mainframe computer. The data were tabulated by the appropriate pathology data system and if sample sizes were adequate, these data were analyzed separately for each sex by Fisher's exact tests. Incidences of lesions and their statistical significance were taken from each of the NO3-generated printouts (stored in the Archives of Toxicology/Pathology in the SEF building) and summarized in Appendix 9.6.1. In addition, tumor incidences were analyzed by a time adjusted analysis based on Peto's method. A detailed description of the statistical methodology used in this study can be found in Section 9.6.

Mortality: The days on test were regarded as censoring times for animals sacrificed on schedule and as true death times for animals that died or were sacrificed moribund. The survival distribution for each group and each sex was determined using Kaplan-Meier estimates. Nonparametric rank tests: Mantel-Cox logrank test for equality and test for linear trend were performed separately for each sex to test for differences between the survival curves of the treatment groups. If significant differences were found, follow-up pairwise comparisons based on these procedures were then performed to compare each treated group versus the control. A detailed description of the statistical methodology used in this study is given in Section 9.2.

APPENDIX B

Additional data are requested from the sponsor as follows:

1. Historical control data. Data obtained from Sprague-Dawley rats for the last five (5) years at Ciba-Geigy Laboratories (Summit, New Jersey) as follows:

   Mammary gland - adenomas, carcinomas and fibro-adenomas for female rats.

   Pituitary gland - adenomas and carcinomas for female rats.

   Kidney - tubular adenomas and carcinomas for male and female rats.

   Adrenal - cortical adenomas for male rats.

   Liver - adenomas and carcinomas for male rats.

   Thyroid - C-cell adenomas and carcinomas for male rats.

2. All available data on the immunocytochemical staining of the pituitary gland for identification of prolactin.

3. Bone marrow determinations for establishing the Myeloid/Erythroid ratio in all dose groups, males and females.

4. Provide justification for the selection of the dose levels used in this study.

5. Specify the purity of Simazine Technical used in the study.

David G Anderson, PhD. 10/31/88
Section 2, Tox. Branch 1 (IRS) (TS-769C).
Secondary reviewer: Marion Copley. DVM. 11/28/88
Section 2, Tox. Branch 1 (IRS) (TS-769C).

DATA EVALUATION REPORT

037449

STUDY TYPE: One Year Chronic Feeding(83-1)/Dog/Simazine/862001.

TOX. CHEM. No.: 740

MRID No.: 406144-02.

TEST MATERIAL: Simazine, tech.

SYNONYMS: 2-Chloro-4,6-bisethylamine-s-triazine.

STRUCTURE:

SPONSOR: Agricultural Division, Ciba-Geigy Corp., P.O. Box 18300, Greensboro, NC 27419.

TESTING FACILITY: Pharmaceutical Div., Ciba-Geigy Corp., 556 Morris Ave., Summit, NJ 07901.

STUDY NO.: A7/17 (MIN 862001), Toxicology/Pathology Report No. 87122.

REPORT TITLE: Simazine Technical: A 52-Week Oral Feeding Study in Dogs.

AUTHOR(S): G C McCormick and J D Green.

REPORT ISSUED: March 28, 1988.

CORE GRADE: Minimum.

CONCLUSIONS: Toxicity was demonstrated at the HDT in males by decrements in body weight gain, variable but reversible decrements in red blood cell counts, hemoglobin concentration, hematocrit, and statistically significant increases in platelet counts. Similar toxicity was demonstrated at the HDT in females by statistically significant larger decrements in body weight gain, and at the MDT and HDT by decrements in the red blood cell counts, hemoglobin concentration, and hematocrit. Slight nominal increases occurred in platelet counts in HDT females. Decrements in body weight gain occurred in one female at the MDT. This decrement was considered to be compound related, although no other effects were noted in this animal. The efficiency of food utilization was apparently decreased in females at the HDT. In males at the HDT the absolute organ weight and organ/brain weight, and organ/bodyweight ratios were apparently increased for the adrenals (130%), kidneys (111%), liver (108%), and decreased

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in the spleen (69%)and thyroid/parathyroid (60%). In females at the HDT adrenals(129%), liver (104%), and thyroid/parathyroid (114%) weights may have been increased. These and other organ weight effects were not reported to be accompanied by any findings at histological examination, and thus, they may have been incidental to the study. The study reported that the NOEL for the study was 20 ppm.

Dose levels administered by ~~gavage~~ were 0. 20, 100, and 1250 ppm or 0, 0.68, 3.4, and 43 mg/kg/day for males, respectively, and 0, 0.76, 3.6, and 45 mg/kg/day for females, respectively.

NOEL: 20 ppm or 0.76 mg/kg/day for females (LDT).
LEL: 100 ppm or 3.6 mg/kg/day for decreased body weight gain, and decreases in RBC, HGB, HCT, and a nominal increase in platelet counts in females. At 45 mg/kg//day in females decreases occurred in body weight gain, and in RBC, HGB, and HCT. At 43 mg/kg/day in males decrements in body weight gain, and variable but reversible decrements in RBC, HGB, and HCT, and increases in platelet counts.

A. MATERIALS:

1. Test compound: Simazine technical, Description white powder. Batch No. FL #840988. Purity: NOT SPECIFIED, but the purity was designated as 97.5% for the same batch #840988 in a submitted report on a 90-day dog study.

2. Test animals: Species: DOG, Strain: Beagle, Age: Approximately 6 months, Weight: Males = 7.5-9.1 kg, females = 6.5-8.2 kg, Source: NOT SPECIFIED. Acclimatization period - 7 weeks.

3. Environmental: Temperature - 69 ± 5°F. Humidity - 50± 20%. Ratio light:dark = 12:12.

B. STUDY DESIGN:

1. Animal Assignment - Animals were assigned randomly to test groups.

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Test Group	Dose in diet ppm	Mean daily dose mg/kg/day		Main study 12 months		Clinical study Pre-dose, wk 14, wk 26, and wk 52	
		Male	Female	Male	Female	Male	Female
1. Cont.	0	0.0	0.0	4	4	4	4
2. Low (LDT)	20	0.68	0.76	4	4	4	4
3. Mid (MDT)	100	3.41	3.64	4	4	4	4
4. High(HDT)	1250	42.9	44.9	4	4	4	4

2. Diet preparation - diet was prepared weekly, and stored at an unspecified temperature. Samples of treated food were analyzed for stability at room temperature over a 40 day period, and concentration at a predetermined frequency specified by statistical design which indicated that analysis should be conducted on 13 of the 52 diet preparations. Diets prepared on weeks 2, 4, 11, 12, 20, 23, 25, 26, 29, 34, 42, 43, and 49 were analyzed at each dose level.

Results - Stability at 40 days was within 3% of the initial concentration. The concentration of the test material in the feed was within 7% of the nominal at all dose levels. Homogeneity was within 2% of nominal. Thus, the stability , concentrations, and homogeneity of the test material in the feed was satisfactory.

3. Animals receive food, Certified Purina Canine Diet #5007, and water ad libitum.

4. Statistics - The following procedures were utilized in analyzing the numerical data: Beckman TOXSYS data base.

5. Quality assurance was signed by George C McCormick, the Study Director on April 5,1988, and James D Green, The Director of Research on April 4, 1988, and Lynn R Miko, The Director of QAU, Regulatory Compliance, on March 1, 1988.

6. METHODS AND RESULTS:

1. Observations - Animals were inspected daily for signs of toxicity and mortality.

Results - Toxicity - Cachexia was observed in 1 high dose male from weeks 14 through 20, and in 1 high dose female from week 14 through week 22. Other observations with dose apparent dose relationship occurred in fecal changes (diarrhea, discoloration, presence of blood, few, mucoid, and soft , and infrequent emesis.

Mortality (Survival) - No unscheduled death occurred during the study period.

2. Special Studies - Included in the physical examination on study weeks 14, 26, 40, and 52 were the recording of abnormal discharges/exudates from body orifices, character of hair coat and attitude, rectal temperature, and heart rate via auscultation of the left thoracic area (beats/15 seconds * 4 = beats per minute).
The hearing of each dog was evaluated by a "clap test".

Results - The heart rate was slightly but statistically significantly increased ($p<0.05$) only in males at the MDT and HDT (no dose related trend was indicated), and only in the determination on study week 40. Temperature was slightly but statistically significantly increased ($p<0.05$) only in males at the MDT and HDT, and only in the determination on study week 26 and week 40 (again no dose related trend was indicated). However, neither the data on the heart rate nor the data on the body temperature demonstrated any pattern of progression with time or dose, and thus they were not considered to be dose related.
It was reported that no effects were noted in auditory segment of these tests, but no data was reported.

3. Body Weight - They were weighed pretest, and weekly for the first 12 weeks, then monthly thereafter.

Results - Male body weight gain was nominally depressed at the HDT during most of the study, but not at the end of the study. Male body weight gain was statistically significantly depressed at the HDT only during the first two weeks of the study. Female body weight was frequently statistically significantly depressed at the HDT for the first 20 weeks of the study, and they lost or failed to gain body weight during this period. Female body weight gain at the HDT was frequently statistically significantly depressed during the first 36 weeks of the study at which time they appeared to start gaining body weight. One female at the MDT lost or failed to gain weight during the first 4-5 weeks of the study, and gained less than half the weight other animals gained at this dose level. See the Appendix, report pages 246 - 249 at the end of this report after Table 8.3. The remaining animals at the MDT gained a normal amount of weight. Table 8.3 presents a summary of the body weight and body weight gain for males and females.

4. Food consumption and compound intake - Consumption was determined and mean daily diet consumption was calculated. Efficiency and compound intake were calculated from the consumption and the body weight gain data.

Results - Food consumption - No significant reductions in food

intake occurred in males, however statistically significant reductions in food consumption occurred in females at the HDT frequently during the first 20 weeks of the study.

Food efficiency - The efficiency of food utilization was not submitted. Table A illustrates values calculated from the submitted report. The efficiency of these animals appears to have compromised only at the HDT in females, and possibly at the MDT in one female, but not for the group, and only for approximately for the first 12 weeks of the study. Since the weight gain pattern for the one female animal was similar to the animals in the HDT, the weight loss was believed to be compound related. The usefulness of Table A is limited because animals demonstrated periodic emesis, and there was no indication that this was accounted for in the total food consumption.

Table A.
Calculated Values for the Relative Efficiency of Food Utilization. Calculations are presented for the First 12 Weeks and the Last 40 Weeks of the Study.

Group	Relative Efficiency for weeks 0 through 12		Relative Efficiency for weeks 13 through 52	
	Males	Females	Males	Females
1. Control	0.034	0.051	0.017	0.0073
2. 20 ppm	0.074	0.061	0.015	0.012
3. 100 ppm	0.061	0.050	0.019	0.0082
4. 1250 ppm	0.032	-0.0048	0.020	0.014

Compound intake - for males was 0.68, 3.41, and 43.0 mg/kg/day and for females 0.76, 3.64, and 44.9 mg/kg/day for the 20, 100, and 1250 ppm dose groups, respectively.

5. Ophthalmological examinations were performed pre-dosing, week 26, and week 52 on all animals.

Results - No remarkable findings were reported from the pre-dosing examination. Corneal opacity occurred in 1 MDT dog at the 26 week examination, which was apparently normal at the 52 week examination, and 1 HDT female demonstrated Lens cortical density at the 52 week examination. All other animals were reported to be normal.

6. Blood was collected before treatment and at day 86, 177, and 359 for hematological and clinical analysis from all animals. The CHECKED (X) parameters were examined.

a. Hematology -

X Hematocrit (HCT)*	Total plasma protein (TP)
X Hemoglobin (HGB)*	X Leukocyte differential count*
X Leukocyte count (WBC)*	X Mean corpuscular HGB (MCH)
X Erythrocyte count (RBC)*	X Mean corpuscular HGB conc. (MCHC)
X Platelet count*	X Mean corpuscular volume (MCV)
Blood Clotting Measurements	X Reticulocyte count, control & HDT
X (Clotting time)	X Heinz bodies
X (Prothrombin time)	

* Required for subchronic and chronic studies

Results - Slight treatment related changes occurred in the hematological parameters, which were less severe in males than in females (See Table B in the Appendix). In males at the HDT, a transient nominal decrease (non significant) in RBC, and HGB at days 86 and 177, while HCT was statistically significantly depressed at 86 days. At the time of the last bleeding on the day 359, these values were nominally higher or equivalent to control values. MCV, MCHB, and MCHC values did not vary more than the standard at any time. The platelets were statistically significantly elevated in the HDT male dogs at the end of 86 (142%), 177 (170%), and 359 (155%) days of dosing.

In females at the HDT, a statistically significant transient decrease in RBC, HGB, and HCT occurred at 86, and 177 days. These values were only nominally depressed at 359 days compared to control dogs, but not when compared to pre-dose values in HDT female dogs. MCV, MCHB, and MCHC values were within the experimental error of the controls, but MCHB was slightly but statistically significantly elevated at the HDT after 359 days of dosing.

b. Clinical Chemistry

Electrolytes:	Other:
X Calcium*	X Albumin*
X Chloride*	X Blood creatinine*
Magnesium*	X Blood urea nitrogen*
X Phosphorus*	X Cholesterol*
X Potassium*	X Globulins
X Sodium*	X Glucose*
ENZYMES:	X Total bilirubin*
X Alkaline Phosphatase (AP)	X Total protein*
Cholinesterase (CHE)=	Triglycerides (TG)
X Creatinine phosphokinase*'(CP)	Serum protein electroph.
Lactic acid dehydrogenase (LDH)	
X Serum alanine aminotransferase* (also SGPT)	
X Serum aspartate aminotransferase* (also SGOT)	
X Gamma glutamyl transferase	
Glutamate dehydrogenase	

* Required for subchronic and chronic studies
= Should be required for OP's
' Not required for subchronic studies

Results - Various apparently random changes in clinical chemistry parameters occurred at the HDT, but these changes were neither consistent nor dose related. CPK was elevated in controls, LDT, and MDT males in data at 86 days, but it had returned to normal in all groups by the end of the study. Alkaline phosphatase was elevated in high dose males at the beginning of the study but had returned to normal for the remainder of the study. Sodium was statistically significantly elevated in the high dose group males at 177 days, but it was normal for all other periods of analysis. Statistically significant decreases in calcium in all dose groups females at 86 days were neither dose related nor present on other days.

7. Urinalysis' - Urine was collected, usually by catheterization, from animals pre-dosing, and at 86, 177, and 359 days. The CHECKED (X) parameters were examined.

Appearance*	X Glucose*
Volume*	X Ketones*
X Specific gravity*	X Bilirubin*
X pH	X Blood*
X Sediment (microscopic)*	Nitrate
X Protein*	X Urobilinogen

* Required for chronic studies
' Not required for subchronic studies

Results - All of the parameters examined did not differ in dose related manner from control values on any examination day or in any dose group.

8. Sacrifice and Pathology -

All animals that died and that were sacrificed on schedule were subject to gross pathological examination. The (X) tissues were collected for histological examination. The (XX) organs, in addition, were weighed.

X DIGESTIVE SYSTEM	X CARDIVASC./HEMAT.	X NEUROLOGIC
Tongue	X Aorta*	XX Brain*
X Salivary glands*	XX Heart*	X Periph nerve*
X Esophagus*	X Bone marrow*	X Spinal cord (3 levels)
X Stomach*	X Lymph nodes*	XX Pituitary*
D Duodenum*	XX Spleen*	X Eyes(optic nerve)
D Jejunum*	XX Thymus*	GLANDULAR
D Ileum*	UROGENITAL	XX Adrenal*
D Cecum*	XX Kidneys*	X Lacrimal gland*
D Colon*	X Urinary bladder*	Mammary gland*
X Rectum*	XX Testes*	XX Parathyroids*
XX Liver*	XX Epididymides*	XX Thyroids*
X Gall bladder*	X Prostate	OTHER
X Pancreas*	Seminal Vesicle	Bone*
RESPIRATORY	XX Ovaries	X Skeletal musc.*
X Trachea*	X Uterus*	X All gross lesions & masses.
X Lungs*	X Vagina	X Cranial nerves
X Large and small intestines		X Skin- mammae

D = In this study these tissues were designated as the large and small intestines (general).

Results -

a. Organ weights - No dose related effects occurred on organ weights in males or females in any group. Increases in males of adrenal (130%), adrenal/brain, and adrenal/body weight ratios, kidney (111%) and kidney/brain, and kidney/body weight ratios, and liver (108%), and liver/brain ratios occurred in the high dose group. The adrenal/brain weight ratio ($p<0.05$) was the only statistically significant organ weight effect in males at the high dose. In males, thyroid/parathyroid (60%) weights, thyroid/parathyroid/brain and body weight ratios, spleen (69%) weights, spleen/brain, and spleen/body weight ratios were all nominally decreased in the high dose group. Only the standard error of the absolute thyroid weights, and thyroid weight ratios were statistically significantly less than ($p<0.001$) control values. In females at the HDT, increases occurred in adrenal

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(129%, $p<0.01$), adrenal/brain ($p<0.01$), and adrenal/body weight ratios ($p<0.01$), thyroid/parathyroid (114%), thyroid/brain, and thyroid/body weight. Spleen (81%) weight, spleen/brain, and spleen/body weight ratios were nominally decreased in females in the high dose group. The adrenal weight, adrenal/brain, and adrenal/body weight ratio at the HDT were the only organ weights and ratios which were consistently statistically significant.

b. Gross pathology - No dose related or compound related gross pathology was noted at any dose level.

c. Microscopic pathology -

1) Non-neoplastic - No dose related or compound related histopathology was noted in any animal in any dose group. Microscopic lesions were found, but no dose related pattern could be detected. Lesions in the high dose group appeared no more frequently than in controls, and thus, no compound related histopathology was detected. None of the organ weight or organ weight ratio increases or decreases was associated with any reported histopathology.

2) Neoplastic - No neoplastic lesions were reported, if detected.

D. DISCUSSION:

Body weight gain at the HDT in males on study day 7, and 14, and in females on study days 7-224 were statistically significantly less than control values. Body weights of females on study days 63 through 140 were statistically significantly less than control values. One female at the MDT lost or failed to gain body weight during the initial weeks of the study, and considering the similar pattern to the females in the HDT, the body weight decrement probably was dose related. However, no other obviously altered parameters were noted in the hematology, clinical chemistry values, or in the histopathology of this female dog. Food consumption was also depressed during these periods, but the efficiency of food utilization could be considered depressed only at the HDT and only in females from weeks 0-12.

Hematological parameters were affected more in females than males. The hematocrit (HCT) was statistically significantly depressed, and red blood cell count (RBC) and hemoglobin concentration (HGB) was nominally depressed in males at the HDT, but not at the end of the study. In females at the MDT and HDT, HCT and HGB were statistically significantly depressed, respectively, and at the HDT, RBC, HGB, and HCT were

statistically significantly depressed. Platelets were statistically significantly elevated in males in blood from the 86, 177, and 359 day bleedings, and nominally elevated in females at the HDT. The mean corpuscular volume, the mean corpuscular hemoglobin concentration, and the mean corpuscular hemoglobin gave no indication of the nature of the hematological effect. All these effects were within the normal variation seen in dogs. The study reported that the hematological effects were secondary to the body weight decrements, however, no additional data were submitted as evidence.

The clinical chemistry values were variable, and some such as sodium elevation in males, and calcium depression in females, may have been treatment related, but none appeared to demonstrate a good dose related response. All are considered incidental to the study in agreement with the report on the study.

Various absolute organ weights, organ/brain, and organ/body weight ratios were elevated, and some depressed, but since none demonstrated any dose related effects on histological examination, all may have been incidental to the study.

Histological examination of the animals organs and tissues did not reveal any dose related effects.

The heart rates in males on week 40 were slightly but statistically significantly ($p<0.05$) elevated at the MDT and HDT. The body temperatures in males on weeks 26 and 40 were slightly but statistically significantly elevated at the MDT and HDT. None of these effects indicated trends with time or any dose related trends, and they were considered to be incidental to the study.

The study was relatively free of toxic effects with the body weight gains and hematological parameters being the only indications of toxicity. Even these effects were minimal, except the body weight effects in the high dose females.

There were deficiencies in this report, and although, they probably do not compromise the study, some of them increased the time required to review the study.

1. Summary tables were not presented for most of the urinalyses data.
2. The hematology data could have been summarized in a manner more easily reviewed.
3. It was difficult to determine which organs and tissues, and the number which were histologically examined. Summary tables could have been more clearly explained and presented, such as required histology on some apparently normal organs and tissues were not specifically reported, e.g. the adrenal, gastrointestinal tract, aorta, pancreas, etc.
4. The source of the animals was not stated.
5. The purity of the test material was not reported.
6. Food efficiency was not calculated.

* Recommended by Subdivision F (Oct. 1982) guidelines for chronic studies.

E. APPENDIX:

[redacted]

One Year Chronic Feeding/Dog/Simazine/862001.

Table B.

Group	Days on Study	Males RBC 10**6/C.MM	HGB GM/DL	HCT %	Retic. %	Platelets 10**3/C.MM	Females RBC 10**6/C.MM	HGB GM/DL	HCT %	Retic. %	Platel 10**3
Controls	Pre-dose	5.97	14.8	44.5	1.7	336	6.33	15.0	44.8	1.1	27
	86	6.51	15.8	45.0	1.0	369	7.28	17.1	49.5	1.1	33
	177	6.32	15.1	44.0	0.5	253	7.24	17.1	49.2	0.9	26
	359	6.54	15.9	46.2	1.0	268	7.00	17.0	49.2	1.2	31
MDT	Pre-dose	5.64	13.6	41.3	2.0	350	6.20	15.3	45.2	0.8	23
	86	6.51	15.4	44.3	-	356	6.29	15.2*	43.5*	-	37
	177	6.79	16.4	46.8	-	257	6.48	15.5	45.0	-	28
	359	7.30	18.4*	51.8	-	246	6.82	17.0	48.8	-	34
HDT	Pre-dose	5.90	14.0	42.0	1.9	360	6.10	15.3	45.0	1.6	31
	86	5.80	13.9	38.8*	1.2	487*	6.04*	14.9*	42.5**	1.5	41
	177	6.18	14.9	42.5	0.8	432**	6.14*	15.0*	43.2*	1.4	37
	359	6.82	16.9	48.2	1.7	416**	6.30	16.0	46.0	1.6	36

* = $p < 0.05$
** = $p < 0.01$

19.c

SIMAZINE

Page_____ is not included in this copy.

Pages 62 through 70 are not included.

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EPA: 68D80056
DYNAMAC No. 1-10A
February 3, 1989

DATA EVALUATION RECORD

SIMAZINE

Chronic Toxicity/Oncogenicity Feeding Study in Mice

APPRCVED BY:

Robert J. Weir, Ph.D.
Program Manager
Dynamac Corporation

Signature: Robert J. Weir

Date: 2-3-89

EPA: 68D80056
DYNAMAC No. 1-10A
February 3, 1989

DATA EVALUATION RECORD

SIMAZINE

Chronic Toxicity/Oncogenicity Feeding Study in Mice

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37449

DATA EVALUATION RECORD

STUDY TYPE: Chronic Toxicity/Oncogenicity Feeding Study in Mice.

ACCESSION/MRID NUMBER: 406144-04.

TEST MATERIAL: Simazine technical.

SYNONYM(S): 2 Chloro-4,6 bis(ethylamino)-s-triazine.

STUDY NUMBER(S): Laboratory Study No. 842121.

SPONSOR: Agricultural Division, Ciba-Geigy Corp., Greensboro, NC.

TESTING FACILITY: Pharmaceuticals Division, Ciba-Geigy Corp., Summit, NJ.

TITLE OF REPORT: Simazine technical; 95-week oral toxicity/oncogenicity study in mice.

AUTHOR(S): Hazelette, JR and JD Green.

REPORT ISSUED: April 4, 1988.

[redacted]

CONCLUSIONS:

Simazine was not oncogenic in CD-1 mice when fed in the diet at concentrations of 40, 1000, or 4000 ppm for 95 weeks. There was a decrease in mean body weight in both males and females in the mid-and high-dose groups, and a decrease in food consumption in mid- and high-dose males and in mid-dose females. There were decreases in erythroid parameters which may have been related to weight loss. Other hematologic parameters were not affected. Clinical chemistry values and urinary parameters were normal in dosed groups. Organ-to-body weight ratios were increased in high-dose females for several organs; however, there were no histologic correlates and the changes were accompanied by decreased terminal body weights. There were no nonneoplastic changes related to dosing. The incidence of amyloidosis was high in all groups. The LOEL based on decreased weight gain was 1000 ppm and the NOEL 40 ppm.

Classification: Core guideline.

A. MATERIALS:

1. Test Compound: Simazine technical; description: white powder; batch No.: FL 840988; purity: not reported.

2. Test Animals: species: mice; strain: Crl:CD1(ICR)BR; age: approximately 5 weeks at initiation; weight: males--19.1 to 32.1 g; females--14.4 to 26.3 g; source: Charles River Breeding Laboratories, Kingston, NY.

B. STUDY DESIGN:

1. Animal Assignment: Animals were acclimated to laboratory conditions for 14 days and were assigned randomly by sex to the following test groups after passing a physical examination:

[redacted]

Test group	Dose in diet (ppm)	Main study (95 weeks)	Satellite groups (Pre)	(26 weeks)	(52 weeks)	(56 weeks)[c]
			Males/Females			
1 Control	0	60	-	10	10	10
2 Low (LDT)	40	60	-	10	10	-
3 Mid (MDT)	1000	60	-	10	10	-
4 High (MDT)	4000	60	-	10	10	10
5 Baseline[a]	0	-	60	-	-	-
6 Sentinel[b]	0	-	-	-	20	-

[a]Used for baseline laboratory values; 30/sex at -1 and at 2 weeks.
[b]Used for viral screen.
[c]Recovery group; received undosed diets from week 52 to 56.

Mice were housed individually in a temperature and humidity controlled room with a 12-hour light/dark cycle.

2. Diet Preparation: Dietary mixtures of test substance at concentrations of 0, 40, 1,000, and 4,000 ppm were prepared and used within 21 days. Stability of test compound stored for 40 days at room temperature in closed amber glass containers was determined. Test Compound in the diets was analyzed at 4 week intervals for 1 year and at approximately 8-week intervals thereafter. Homogeneity was determined at weeks 1, 58 (high-dose), and 68.

   Results: The diets were homogeneous; the standard deviations as percent ranged from 0.2 to 2.3 percent for samples at 3 levels. Test material was stable in diets; 95 and 99% was recovered after 40 days storage at room temperature, at dietary levels of 40 and 4,000 ppm, respectively. All diets were within 8 percent of target. Table 1 presents representative analytical data.

3. Food and Water Consumption: Animals received food (Purina Rodent Chow No. 5002) and water ad libitum.

4. Statistics: The following procedures were utilized in analyzing the numerical data:

   Body weights, food consumption, clinical pathology, and organ weights were analyzed by Bartlett's test for equality of variances. If variances were homogeneous, Dunnett's test was used to compare control versus each dose group. Rank transformations or nonparametric tests were used when variances were not homogeneous. Survival data were analyzed using Kaplan-Meir estimates. The generalized Wilcoxon test for equality and the Mantel-Cox log-rank test

were used for group comparisons. Pathology data were analyzed separately by sex using the Fisher exact test. In addition, tumor incidence was analyzed by time-adjusted analysis based on the Peto method.

TABLE 1. Analysis of Simazine in Test Diets at Representative Intervals

		Target Concentration (ppm)		
		40	1,000	4,000
Week				
1	Concentration (ppm)	37.5	999.0	3719
	Percentage of target	94	100	93
24	Concentration (ppm)	38.7	964.0	3970
	Percentage of target	97	96	99
52	Concentration (ppm)	40.3	1022	3952
	Percent of target	101	102	99
92	Concentration (ppm)	38.8	1030	4145
	Percentage of target	97	103	104

5. Quality Assurance: A quality assurance statement was signed and dated April 4, 1988.

C. METHODS AND RESULTS:

1. Observations: Animals were inspected twice daily for mortality and moribundity (once daily on weekends). Animals received detailed physical examinations, including palpations at initiation and at 2-week intervals during the study.

Results: There were no effects of dosing on the incidence of clinical signs. The most frequent observations were corneal opacity, cachexia, polyuria (males) and fur staining. Summary incidence data (observation in any animal in a group at any study interval) and group incidence for each type of observation at weekly intervals were presented. Examination of the latter tabulation (CBI Report, Table 8.4) indicated that all observations were incidental. There was a fairly high incidence of corneal opacity in control and high-dose animals at various intervals of the study. This may have been caused by periorbital bleeding for clinical pathology but this could not be verified in the absence of individual findings.

The initial viral screen on the sentinel animals indicated the presence of antibodies to MMV (mouse minute virus) but none of the other viruses tested positive. Since MMV was found in controls as well as dosed groups and since it did not adversely affect survival, it is not considered a serious consequence in the health of the mice.

There was no significant effect of dosing on survival. Table 2 presents data on mortality and survival.

TABLE 2. Cumulative Mortality and Percent Survival in Mice Fed Simazine for 95 Weeks

Dietary level (ppm)	No. of animals initial	No. of animals termination	No. of mortalities and (percent survival) at week 52	78	96
		MALES			
0	90	19	3(96)[a]	34(46)[b]	44(30)[b]
40	80	15	1(98)	32(50)	47(24)
1000	80	13	1(98)	35(43)	48(21)
4000	90	15	2(97)	28(54)	48(25)
		FEMALES			
0	90	20	3(96)	17(72)	35(43)
40	80	26	4(94)	21(65)	34(43)
1000	80	35	4(94)	14(76)	24(60)
4000	90	25	5(93)	17(72)	36(42)

[a]Percent survival was based on 80, 71, 70, and 80 males and 80, 70, 70, and 80 females at 0, 40, 1000, and 4000 ppm; 9 to 10 animals/group were sacrificed at 26 weeks.

[b]Percent survival based on 63, 62, 61, and 61 males and 61, 60, 60 and 61 females at 0, 40, 1000, and 4000 ppm; 9 to 10 animals were sacrificed at week 52 in all groups and at week 56, 8 control and 9 high-dose males and 10 control and 9 high-dose females in the recovery segment were sacrificed. These values differ slightly from Table 8.1 of the report which based survival on the total number of animals minus the animals scheduled for interim sacrifice.

2. Body Weight: Mice were weighed weekly from 1 week prior to initiation to week 13 and monthly from week 16 to study termination.

Results: Table 3 presents representative data on mean body weights in males and females. There was a significant reduction of mean body weights and percent weight gain in males and females receiving 1000 ppm and 4000 ppm. The reductions at the highest dose were significant throughout most of the study and at the mid dose the reductions were significant in males beginning at week 24 and in females beginning at week 16. The mean body weights of males receiving 40 ppm were slightly but significantly ($p < 0.05$ decreased at 4 study intervals (44, 56, 60, and 64 weeks). These were not considered of toxicological significance by

the study authors because they were isolated occurrences. At the termination of the 4-week recovery period, the mean body weight in the group of males that had previously received 4000 ppm simazine (42.7 g) did not differ significantly from controls (39.8 g) but in recovery females the mean body weights still remained depressed in the group previously received 4000 ppm simazine (27.8 g compared to 38.6 g).

TABLE 3. Representative Results of Mean Body Weights of Mice Fed Simazine Technical For 95 Weeks

Dose group (ppm)	Mean body weights (g ± S.E.) at day					
	0	7	140	392	504	644
			MALES			
0	23.9 ± 0.18	26.7 ± 0.20	38.6 ± 0.39	42.5 ± 0.61	42.6 ± 0.95	41.3 ± 1.65
40	24.2 ± 0.22	27.2 ± 0.23	38.7 ± 0.47	40.5 ± 0.54*	40.8 ± 0.64	39.3 ± 1.66
1000	23.9 ± 0.22	26.6 ± 0.24	36.9 ± 0.43**	39.3 ± 0.57**	38.2 ± 0.74**	38.8 ± 1.33
4000	24.2 ± 0.19	25.9 ± 0.21*	34.8 ± 0.30**	36.8 ± 0.40**	36.8 ± 0.45**	36.3 ± 0.99*
			FEMALES			
0	20.0 ± 0.17	21.9 ± 0.16	32.5 ± 0.40	36.6 ± 0.63	37.4 ± 0.71	37.5 ± 1.88
40	20.3 ± 0.20	21.8 ± 0.18	32.4 ± 0.39	36.6 ± 0.64	36.9 ± 0.65	37.1 ± 1.98
1000	20.2 ± 0.18	21.5 ± 0.17	30.2 ± 0.27**	33.7 ± 0.42**	34.2 ± 0.50**	34.4 ± 1.60**
4000	20.5 ± 0.17	20.8 ± 0.16**	27.9 ± 0.22**	29.2 ± 0.32**	30.4 ± 0.41**	30.3 ± 1.52**

*Significantly different from control values at $p < 0.05$.
**Significantly different from control values at $p < 0.01$.

3. Food Consumption and Compound Intake: Consumption was determined and mean daily diet consumption was calculated at the same intervals as the weighings. Compound intake was calculated. Water consumption was measured for 5 days at weeks 1, 2, 52, 53, 92, and 93.

Results: Mean food consumption was decreased compared to controls in males and females receiving 4000 ppm and in males receiving 1000 ppm. The decreases were significant at most intervals to 84 weeks. Table 4 summarizes representative data. In the recovery groups, the food consumption also correlated with body weight gains; it was decreased compared to controls in the females but not males that had previously received 4000 ppm simazine. Mean compound intake for the entire study was 5.3, 131.5, and 542 mg/kg/day for males receiving 40, 1000, and 4000 ppm, respectively; for females at those doses intake was 6.2, 160.0 and 652.2 mg/kg/day, respectively.

TABLE 4. Representative Food Consumption for Mice Fed Simazine Technical for 95 Weeks

Dose group (ppm)	Mean food consumption (gm/week ± S.E.) at day 7	14	84	196	366	644
			MALES			
0	48.1 ± 0.76	44.8 ± 0.47	43.0 ± 0.82	34.0 ± 0.43	28.9 ± 0.37	33.3 ± 1.3
40	48.9 ± 0.86	48.0 ± 0.56*	43.3 ± 0.82	32.8 ± 0.44	28.2 ± 0.45	32.4 ± 1.1
1000	48.5 ± 0.75	39.7 ± 0.63**	39.4 ± 0.62**	31.7 ± 0.47**	27.6 ± 0.36*	32.4 ± 1.4
4000	47.4 ± 0.84	38.7 ± 0.51**	41.5 ± 0.87	30.2 ± 0.35**	27.4 ± 0.34**	31.2 ± 0.9
			FEMALES			
0	45.2 ± 0.99	43.07 ± 0.52	47.7 ± 0.87	33.9 ± 0.70	29.9 ± 0.59	32.6 ± 0.88
40	46.5 ± 0.80	44.9 ± 0.70	46.9 ± 0.90	34.1 ± 0.77	28.7 ± 0.51	32.2 ± 0.82
1000	47.4 ± 0.97	43.8 ± 0.79	44.3 ± 0.86*	32.2 ± 0.78	28.1 ± 0.61	32.4 ± 0.79
4000	44.1 ± 0.68	36.1 ± 0.38**	44.5 ± 0.76*	30.9 ± 0.75*	27.9 ± 0.60*	29.6 ± 0.72*

*Significantly different from control values at p <0.05.
**Significantly different from control values at p <0.01.

Water consumption tended to be decreased in mid- and high-dose males and females (Table 5).

TABLE 5. Representative Water Consumption for Mice Fed Simazine Technical for 95 Weeks

Dose group (ppm)	Mean water consumption (gm/week ± S.E.) at week 1	2	52	92
		MALES		
0	40.1 ± 2.3	45.2 ± 2.1	33.6 ± 2.2	43.3 ± 4.3
40	41.6 ± 2.4	48.1 ± 2.5	35.1 ± 3.6	34.2 ± 5.0
1000	35.2 ± 2.6	38.1 ± 3.0	29.9 ± 3.5	34.4 ± 3.3
4000	32.3 ± 1.8	35.1 ± 2.6**	28.9 ± 2.4	33.4 ± 3.2
		FEMALES		
0	35.4 ± 2.1	36.3 ± 1.8	38.5 ± 5.2	43.3 ± 5.4
40	36.9 ± 2.2	35.5 ± 2.1	31.0 ± 3.2	33.7 ± 3.1
1000	30.5 ± 1.3	31.7 ± 1.7	30.1 ± 3.9	30.4 ± 2.3
4000	28.5 ± 1.5*	23.4 ± 1.1**	21.7 ± 2.3**	26.9 ± 3.7*

*Significantly different from control values at p <0.05.
**Significantly different from control values at p <0.01.

4. Ophthalmological Examinations: Ophthalmological examinations were performed on all animals prior to initiation and all survivors at week 52 and prior to termination (week 96). Examination was also performed on mice in the recovery groups prior to sacrifice (week 56) and on 3 to 6 males/group and 5 to 9 females/group at week 78.

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Results: There were no abnormalities at the predose examination. There were no apparent increases in the incidence of findings in dosed groups when compared to controls. Table 6 summarizes findings at weeks 52 and 95.

TABLE 6. Representative Ophthalmologic Findings in Mice Fed Simazine Technical for 95 Weeks

Finding	Dose group (ppm) 0	40	1000	4000
	Week 52			
Corneal opacity				
Males	20/77[a]	10/67	13/67	18/75
Females	7/76	4/66	10/66	7/54
	Week 95			
Corneal opacity				
Males	5/19	8/15	1/13	3/15
Females	2/27	1/26	3/36	1/29
Cataract				
Males	6/19	10/15	2/13	10/15
Females	18/27	10/26	17/36	17/29

[a]The numerator is the number of animals with the finding and the denominator the number examined.

5. Hematology and Clinical Chemistry: Blood was collected from the periorbital sinus prior to study initiation and at 6 and 12 months for hematology and clinical analysis from 10 animals/sex/group and prior to termination on all survivors. An additional group of 60 mice/sex were sacrificed during week -1 and 2 to obtain baseline clinical laboratory values. The CHECKED (X) parameters were examined:

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a. Hematology

X Hematocrit (HCT)*
X Hemoglobin (HGB)*
X Leukocyte count (WBC)*
X Erythrocyte count (RBC)*
X Platelet count*
X Reticulocyte count (RETIC)
X Red cell morphology
X Leukocyte differential count
X Mean corpuscular HGB (MCH)
X Mean corpuscular HGB concentration (MCHC)
X Mean corpuscular volume (MCV)
X Coagulation:thromboplastin time (PT)-(baseline only)

Blood smears were prepared for all animals that were sacrificed moribund for differential white cell counts and microscopic evaluation of red cell morphology.

Results: Table 7 summarizes selected data on hematology. Erythrocyte counts (RBC) tended to be decreased in the high-dose groups at all intervals. The decreases were slight and values were not consistently significant at all intervals. Hematocrit (HCT) and hemoglobin (HGB) values tended to be decreased at the high dose but the values were only significant for HCT for high-dose males at 184 day and for HGB in high-dose females at 365 days. There were no clear cut dose-related trends and the changes in erythroid indices (MCV, MCHC) did not correlate with changes in RBC, HCT and HGB. Slight alterations in other hematologic parameters were not considered of any biologic importance. No Heinz bodies were found. Data on blood smears for animals sacrificed moribund were not useful because of frequent technical problems and poor smears. Only a few slides could be evaluated. Baseline data were not reported.

*Recommended by Subdivision F (October 1982) Guidelines.

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TABLE 7. Selected Hematology Parameters (Mean ± S.E.) in Male Rats Fed Simazine Technical for 95 Weeks

Parameter/Interval	Dietary level (ppm) 0	40	1000	4000
MALES				
RBC ($10^3/mm^3$)				
184 days	8.98 ± 0.20	8.11 ± 0.28	7.57 ± 0.28**	8.11 ± 0.27*
365 days	8.10 ± 0.23	7.68 ± 0.54	7.89 ± 0.22	7.81 ± 0.18
667 days	6.63 ± 0.21	6.95 ± 0.20	6.64 ± 0.18	5.96 ± 0.15*
HGB (g/dL)				
184 days	15.50 ± 0.37	15.30 ± 0.24	14.68 ± 0.22	14.55 ± 0.30
365 days	15.16 ± 0.30	14.18 ± 0.85	14.39 ± 0.34	14.62 ± 0.25
667 days	12.94 ± 0.47	13.73 ± 0.34	13.46 ± 0.36	12.23 ± 0.33
HCT (%)				
184 days	48.60 ± 1.08	47.00 ± 0.98	45.70 ± 0.83	44.30 ± 0.82*
365 days	45.67 ± 0.78	43.11 ± 2.16	43.22 ± 0.91	43.50 ± 0.91
667 days	39.74 ± 1.45	41.69 ± 1.04	41.15 ± 1.32	37.13 ± 0.82
FEMALES				
RBC ($10^3/mm^3$)				
184 days	8.04 ± 0.33	8.64 ± 0.18	8.30 ± 0.23	7.76 ± 0.32
365 days	8.86 ± 0.46	8.45 ± 0.29	7.82 ± 0.21	7.77 ± 0.24*
667 days	6.46 ± 0.27	7.14 ± 0.16	5.89 ± 0.17	5.83 ± 0.17
HGB (g/dL)				
184 days	15.84 ± 0.28	15.41 ± 0.17	15.57 ± 0.24	15.24 ± 0.27
365 days	16.98 ± 1.52	15.43 ± 0.29	14.38 ± 0.29	14.15 ± 0.31*
667 days	13.42 ± 0.50	14.26 ± 0.20	12.43 ± 0.35	12.43 ± 0.25
HCT (%)				
184 days	47.70 ± 0.72	47.80 ± 0.47	47.00 ± 0.58	46.00 ± 0.54
365 days	50.22 ± 3.70	45.50 ± 0.85	42.60 ±0.79	41.80 ± 0.76
667 days	41.50 ± 1.51	43.50 ± 0.62	38.24 ± 1.10	37.92 ± 0.65

*Significantly different from control values at $p < 0.05$.
**Significantly different from control values at $p < 0.01$.

b. Clinical Chemistry

Electrolytes	Other
X Calcium*	X Albumin*
X Chloride*	X Albumin/globulin ratio
Magnesium*	Blood creatinine*
X Phosphorus*	X Blood urea nitrogen*
X Potassium*	X Cholesterol*
X Sodium*	X Globulins
	X Glucose*
Enzymes	X Total bilirubin*
X Alkaline phosphatase (ALP)	Direct bilirubin
Cholinesterase	X Total protein*
X Creatinine phosphokinase*	Triglycerides
X Lactic acid dehydrogenase	
X Serum alanine aminotransferase (SGPT)*	
X Serum aspartate aminotransferase (SGOT)*	
X Gamma glutamyltransferase (GGT)	
Urea	

Results: There were no compound-related changes in any serum chemistry parameter. A few values that were significantly different from controls were sporadic, not consistent between intervals of analysis or dose-related, and were marginally changed and within the range of the concurrent controls. These changes included an increase in albumin and chloride in mid- and high-dose females at day 184 and a decrease in LDH in mid-dose females at day 365.

6. Urinalysis: Urine was collected from 10 animals/sex/group at 27, 53, and 96 weeks and from control and high-dose animals in the recovery groups at the beginning of week 57.

X Appearance*	X Glucose*
X Volume*	X Ketones*
X Specific gravity*	X Bilirubin*
X pH	X Blood
Sediment (microscopic)*	Nitrate
Protein*	X Urobilinogen

*Recommended by Subdivision F (October 1982) Guidelines.

Results: There were no compound-related changes in any urinary parameters.

7. Sacrifice and Pathology: All animals that died and that were sacrificed on schedule were subject to gross pathological examination and the CHECKED (X) tissues were collected for histological examination. In addition, the (XX) organs were weighed; (F designates organs weighed after fixation in formalin):

Digestive System	Cardiovasc./Hemat.	Neurologic
X Tongue	X Aorta*	XX Brain*
X Salivary glands*	XX Heart*	X Peripheral nerve (sciatic nerve)*
X Esophagus*	X Bone marrow*	X Spinal cord (3 levels)
X Stomach*	X Lymph nodes*	X Pituitary*
Duodenum*	XX Spleen*	
X Jejunum*	X Thymus*	
X Ileum*	X Eyes (optic nerve)*	
X Cecum*	Urogenital	Glandular
X Colon*	FXX Kidneys*	FXX Adrenals*
X Rectum*	X Urinary bladder*	Lacrimal gland
XX Liver*	FXX Testes*	X Mammary gland*
X Gallbladder*	X Epididymides	FXX Thyroids/ parathyroids*
X Pancreas*	X Prostate	Harderian glands
	X Seminal vesicle	
Respiratory	FXX Ovaries	
X Trachea*	XX Uterus*	Other
XX Lung*	X Vagina	X Bone (sternum)*
X Larynx/pharynx		X Skeletal muscle*
		X Skin
		X All gross lesions and masses

With the exception of one tissue mass, tissues from animals sacrificed at week 26 were not examined. Histopathologic examinations were performed on all animals that died or were sacrificed moribund or were sacrificed by design after 52, 56, and 96 weeks.

*Recommended by Subdivision F (October 1982) Guidelines.

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Results:

a. Organ weights: There were no significant changes in organ weights or organ-to-body or organ-to-brain weight ratios in males after 26, 52 weeks or at the terminal sacrifice with the exception that the heart-to-body weight ratio was increased in high-dose males at 26 weeks. There were several significant ($p = 0.05$ or 0.01) increases in organ-to-body weight ratios in females receiving 1000 and 4000 pm. These changes were generally correlated with reductions of body weights and were not accompanied by increases in absolute organ weights or organ-to-brain weight ratios. Table 8 summarizes data for brain, kidney, and liver weights. Weight changes in heart, adrenal, and lungs were not consistent with time or dose.

b. Gross finding: There were no increases in the incidence of gross findings related to dosing.

c. Microscopic Pathology:

1) Nonneoplastic: Table 9 summarizes frequently occurring lesions in mice that died, were sacrificed moribund, or sacrificed by design after 52 or 95 weeks. Amyloidosis in several tissues showed statistically significant increases in dosed groups. When the number of mice from each group with amyloidosis at any site was compared there was no increase related to dosing. The incidence was fairly high as early a the 52-week sacrifice (62% of males and 20% of females in all groups combined). Incidence of amyloidosis is summarized in Table 10. Amyloidosis was not considered to be related to dosing with simazine.

2) Neoplastic: Table 11 summarizes neoplastic findings. There were no increases in dosed groups in any neoplasm.

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TABLE 8. Mean Organ Weights (± S.E.) and Organ-to-Body Weight Ratios in Female Mice Fed Simazine Technical for 95 Weeks

Organ/Interval	Dietary Level (ppm) 0	40	1000	4000
Brain				
Week 26 (g)	0.516 ± 0.008	0.512 ± 0.013	0.525 ± 0.008	0.499 ± 0.012
(% b.wt.)	1.70 ± 0.09	1.68 ± 0.05	1.98 ± 0.06	2.07 ± 0.04**
Week 52 (g)	0.493 ± 0.006	0.531 ± 0.017	0.536 ± 0.011	0.0525 ± 0.019
(% b.wt.)	1.50 ± 0.07	1.57 ± 0.6	1.86 ± 0.04**	1.93 ± 0.05**
Week 95 (g)	0.550 ± 0.009	0.535 ± 0.007	0.551 ± 0.013	0.510 ± 0.009*
(% b.wt.)	1.72 ± 0.06	1.70 ± 0.05	1.87 ± 0.06	2.009 ± 0.045**
Kidneys				
Week 26 (g)	0.431 ± 0.014	0.447 ± 0.010	0.429 ± 0.011	0.417 ± 0.020
(% b.wt.)	1.41 ± 0.05	1.46 ± 0.04	1.61 ± 0.04**	1.72 ± 0.05**
Week 52 (g)	0.500 ± 0.023	0.465 ± 0.007	0.454 ± 0.018	0.498 ± 0.030
(% b.wt.)	1.50 ± 0.05	1.38 ± 0.05	1.55 ± 0.06	1.82 ± 0.06**
Week 95 (g)	0.553 ± 0.033	0.554 ± 0.015	0.499 ± 0.010	0.425 ± 0.013**
(% b.wt.)	1.71 ± 0.10	1.75 ± 0.06	1.68 ± 0.04	1.66 ± 0.03
Liver				
Week 26 (g)	1.30 ± 0.05	1.31 ± 0.03	1.32 ± 0.06	1.24 ± 0.05
(% b.wt.)	4.22 ± 0.12	4.31 ± 0.12	4.91 ± 0.10**	5.14 ± 0.17**
Week 52 (g)	1.40 ± 0.07	1.38 ± 0.04	1.40 ± 0.05	1.46 ± 0.10
(% b.wt.)	4.19 ± 0.16	4.08 ± 0.13	4.78 ± 0.14*	5.29 ± 0.17**
Week 95 (g)	1.92 ± 0.18	1.55 ± 0.04	1.62 ± 0.05	1.42 ± 0.06**
(b.wt.)	5.90 ± 0.50	4.86 ± 0.14	5.45 ± 0.18	5.54 ± 0.13

*Significantly different from control value, p ≤0.05.
**Significantly different from control value, p ≤0.01.

TABLE 9. Nonneoplastic Findings Frequent in Mice Fed Simazine[a] Technical in the Diet for 95 Weeks

Organ/Findings	Dose level (ppm)							
	Males				Females			
	0	40	1000	4000	0	40	1000	4000
Adrenals	(68)[b]	(66)	(68)	(69)	(69)	(70)	(70)	(69)
Amyloid	33	36	42	34	15	18	14	21
Spindle cell hyperplasia	23	17	15	12	45	46	44	49
Bone marrow	(71)	(70)	(70)	(71)	(70)	(70)	(70)	(71)
Myeloid hyperplasia	9	6	3	7	0	4	2	2
Heart	(71)	(70)	(70)	(71)	(70)	(70)	(69)	(70)
Amyloid	39	29	44	38	9	9	2	16*
Thrombosis	10	7	7	10	3	3	3	2
Intestine, small	(70)	(69)	(69)	(70)	(70)	(70)	(69)	(70)
Amyloid	47	46	51	42	32	30	29	22
Kidney	(71)	(70)	(70)	(71)	(70)	(70)	(70)	(71)
Amyloid	44	47	48	39	25	27	19	19
Mononuclear cell foci	6	5	8	8	5	4	10	1
Liver	(71)	(70)	(70)	(71)	(70)	(70)	(70)	(71)
Amyloid	29	28	40*	32	11	15	13	14
Lungs	(71)	(70)	(70)	(71)	(70)	(70)	(70)	(71)
Amyloid	10	4	7	3	1	3	2	0
Histocytosis	5	3	3	1	6	10	5	3
Lymph node	(58)	(66)	(57)	(53)	(66)	(66)	(65)	(58)
Amyloid	19	19	19	22	5	8	10	12*
Hematopoiesis	7	1	3	12	5	2	2	0
Ovaries					(68)	(68)	(66)	(67)
Amyloid					18	16	10	13
Cyst(s)					22	26	18	18
Salivary glands	(71)	(70)	(70)	(71)	(70)	(69)	(68)	(71)
Amyloid	12	16	24**	13	2	5	4	9*
Spleen	(70)	(70)	(70)	(70)	(70)	(69)	(69)	(71)
Amyloid	7	14	11	11	4	11*	5	4
Hyperplasia	9	5	3	12	6	5	3	5

(continued)

Stomach								
Amyloid	8	6	14	6	5	4	2	2
Testes	(71)	(68)	(70)	(70)				
Amyloid	24	18	29	29				
Thyroid	(68)	(68)	(66)	(67)	(60)	(64)	(66)	(67)
Amyloid	22	26	28	24	7	15*	12	16*
Uterus					(70)	(70)	(70)	(70)
Amyloid					1	2	2	9**

[a]Does not include animals in the recovery group sacrificed after 56 weeks.
[b]The numbers in parentheses are the number of tissues examined histologically.
*Significantly different from control values at $p < 0.05$.
**Significantly different from control values at $p < 0.01$.

TABLE 10. Incidence of Mice with Amyloidosis in Simazine Feeding Study

Dose level (ppm)							
Males				Females			
0	40	1000	4000	0	40	1000	4000
52 Weeks							
8/11	6/10	7/10	5/11	2/10	0/10	4/10	2/11
95 Weeks							
48/60	49/60	52/60	45/60	37/60	34/60	28/60	28/60

[redacted]

TABLE 11. Neoplastic Findings in Mice Fed Simazine Technical for 96 Weeks

Organ/Neoplasm	Dietary level (ppm)							
	Males				Females			
	0	40	1000	4000	0	40	1000	4000
Eye	(70)[a]	(69)	(69)	(69)	(68)	(69)	(70)	(71)
Harderian carcinoma	0	0	0	0	0	0	1	1
Liver	(71)	(70)	(70)	(71)	(70)	(70)	(70)	(71)
Hemangioma/hemangiosarcoma	0	1	0	1	0	0	0	0
Hepatocarcinoma	6	4	2	1	0	1	1	0
Hepatocellular adenoma	1	1	1	1	0	0	0	0
Lungs	(71)	(70)	(70)	(71)	(70)	(70)	(70)	(71)
Adenocarcinoma	3	4	4	3	2	4	3	2
Adenoma	4	3	2	6	6	4	4	5
Ovary					(68)	(70)	68	70
Adenocarcinoma					0	0	0	1
Adenoma					0	1	1	1
Luteal cell tumor, benign					0	0	2	1
Luteal cell tumor, malignant					0	0	1	0
Pituitary	(54)	(55)	(53)	(51)	(57)	(57)	(57)	(59)
Adenocarcinoma	0	0	0	0	0	0	0	0
Adenoma	0	0	0	1	3	0	1	0
Stomach	(70)	(70)	(70)	(71)	(70)	(70)	(70)	(71)
Carcinoma	0	0	0	0	0	0	0	1
Systemic	(71)	(70)	(70)	(71)	(70)	(70)	(70)	(71)
Lymphoma, malignant	1	2	1	3	11	7	8	6
Leukemia	0	1	0	0	1	2	3	3
Histiocytic sarcoma	1	1	0	0	5	4	3	2
Testis	(71)	(68)	(70)	(71)				
Interstitial cell tumor	2	2	0	0				
Uterus					(70)	(70)	(70)	(70)
Adenocarcinoma					1	3	1	0
Adenoma					0	0	2	0
Endometrial stromal sarcoma					0	0	1	1
Hemangioma/hemangiosarcoma					2	4	2	1
Sarcoma (nonspecific)					0	1	0	0

[a]The values in parentheses are the number of tissues examined histologically; includes animal that died, were sacrificed moribund or were sacrificed by design after 52 and 95 weeks.

D. STUDY AUTHORS' CONCLUSIONS:

Under the conditions of the study, simazine technical was not oncogenic in CD-1 mice when administered in the feed at concentrations of 0, 40, 1000, or 4000 ppm for 95 weeks. Amyloidosis and/or intracardiac thrombosis were the major causes of death and moribundity. These lesions were considered incidental since they were found at approximately the same incidence in dosed and control mice. There was no evidence of a compound-related effect on survival or target organ toxicity. Reduced body weights, food and water consumption were found in mid- and high-dose groups. Erythroid parameters and organ weight alterations were found in the same groups. Based on reductions of 14 and 19 percent in body weight gain in males and females, the maximum tolerated dose (MTD) was considered to be 1000 ppm and the NOEL 40 ppm.

E. REVIEWERS' DISCUSSION AND INTERPRETATION OF RESULTS:

The study protocol was acceptable for a chronic toxicity/oncogenicity study in mice. The conduct and reporting of the study were adequate. Sufficient blood was not available for measurement of all the clinical chemistry parameters. This is to be expected in a mouse study.

We assess that the decreased mean weight of mid- and high-dose males and females as well as a decrease in weight gain establish a maximum tolerated dose. The decrease in weight gain correlated with decreased food and water consumption. A decrease in mean body weights noted at four intervals in low-dose males probably indicates a threshold level for an effect. We agree with the study authors' assessment that the decreases were not of toxicologic importance; they were less than 4% of the body weight and there were no corresponding effects in females. The effects of dosing on hematology parameters were not severe and were of doubtful toxicologic importance. Organ weight changes in females were associated with decreased terminal body weights and their importance is doubtful in the absence of any gross or histological correlates.

The incidence of malignant lymphoma was higher in control females than in dosed groups. All values, however, were within the range of incidence found in other laboratories for this strain of mouse. The historical incidence in the testing laboratory was not provided.

We agree with the study authors conclusions that the NOEL was 40 ppm and that there was no oncogenic effect under the conditions of this study.

Secondary reviewer: Marion Copley, DVM.
Section 2, Tox. Branch (IRS)(TS-769C).

DATA EVALUATION REPORT

STUDY TYPE: Teratology/Rat/Simazine/822099.

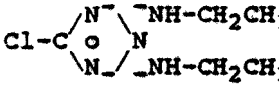
TOX. CHEM. No.: 740

MRID No.: 406144-03.

TEST MATERIAL: Simazine, tech.

SYNONYMS: 2-Chloro-4,6-bisethylamine-s-triazine.

STRUCTURE:



SPONSOR: Agricultural Division, Ciba-Geigy Corp., P.O. Box 18300, Greensboro, NC 27419.

TESTING FACILITY: Pharmaceutical Div., Ciba-Geigy Corp., 556 Morris Ave., Summit, NJ 07901.

STUDY NO.: B6/110 (MIN 822099), Toxicology/Pathology Report No. 83058.

REPORT TITLE: Simazine Technical, a Teratology Study in Rats.

AUTHOR(S): J Mainiero, K Wimbert, J Wright, R N Infurna, A T Arthur, and E T Yau.

REPORT ISSUED: April 7, 1986.

CORE GRADE: Supplementary because additional information must be submitted. See section E.

CONCLUSIONS:
Dose levels administered by gavage were 0, 30, 300, and 600 mg/kg/day. Test animals: Rats-CR1. COBS CD SD BR.

Developemental (Embryo/fetal) toxicity:
NOEL: 30 mg/kg/day.
LEL: 300 mg/kg/day and higher for increased head incompletely ossified, teeth not ossified, centra/vertebrae unossified and/or (additional), rudimentary ribs, presphenoid not ossified, and sternebrae not ossified. No malformations were reported.

Maternal toxicity:
NOEL: 30 mg/kg/day.
LEL: 300 mg/kg/day and higher for decreased maternal body

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weight and body weight gain, food consumption, and efficiency of food utilization.

A/D Ratio = 1.

A. MATERIALS:

1. Test compound: Simazine technical, Description white powder. Batch No.FL-821846, Purity NOT SPECIFIED. The purity was 97.5% and was determined from a report submitted on a 90-day dog study on the same batch of test material.

2. Test animals: Species: Rats, Strain: CR1. COBS CD SD BR. Age: At mating NOT SPECIFIED, Weight: 200-350 g, Source: NCT SPECIFIED. Acclimatization: 7 Days.

B. STUDY DESIGN:

1. Environmental Conditions - Temperature was 22 ±3°C. Humidity was 50% ±20%. Light:dark = 14:10. Eight air changes per hour. Animals were caged individually, except during breeding.

2. Animal Assignment and Breeding - Assignment was by random selection to 4 groups. Breeding was natural with 2 females per male, and gestational day (gd) 0 was the day sperm was detected. The study was initiated on 1/3/83 and terminated on 1/21/83.

3. Test Substance Administration: Test substance was administered by gavage with 2% caboxymethylcellulose as the vehicle. Total volume of the dose was 10 ml/kg. The test substance was administered on gd 6 through gd 15.

Test group	Dose mg/kg/day	Dosage conc. mg/ml	Volume of Doses ml/kg/day	Number of Females
	2% methyl-cellulose			
1. Cont.	vehicle	0.0	10	25
2. Low (LDT)	30	3.0	10	25
3. Mid (MDT)	300	30	10	25
4. High(HDT)	600	60	10	25

4. Analysis of Dosing Solutions: Analyses of dosing suspensions were not reported, but were said to be the responsibility of the sponsor. The frequency of preparation of the test material was not reported. No indication of any preparation of the test material was presented. No stability studies on the test material or dosing suspensions were submitted, but were stated to be the responsibility of the sponsor.

#5002 Certified Chow. The water used was not specified. Both food and water were supplied ad libitum.

6. Statistics - Parametric analysis was conducted on body weight, body weight gain, feed consumption, and fetal weight. Other tests conducted were Test for Outliers (Pearson and Hartely, 1966) and Bartlett's Test for Homogeneity of Variance (Snedecor and Cochran, 1968); for Homogeneous Variances - One-way Analysis of Variance (Snedecor and Cochran, 1968), with Dunnett's Method of Multiple Comparisons (Dunnett, 1964), for Heterogeneous Variances - Behren's T-Test with Cochran's Approximation (Cochran, 1964).

Nonparametric Analysis was conducted on the number of corpora lutea, implantations, resorption sites, viable fetuses, calculated pre-implantation loss, % pre-implantation loss, and % post-implantation loss. Methods were Dunn's Method of Multiple Comparisons Using Rank Sums and (Dunn, 1964) Rank Analysis of Covariance (Quade, 1967).

7. Quality assurance was signed by Robert N Infurna, Study Director, Edward T Yau, Assistant Director, and the sponsor between 4/7/86 and 4/15/86, and Lynn R Miko, Director Regulatory Compliance - OAU, April 4, 1984.

C. METHODS AND RESULTS: Numbered tables were coppied from the submitted report, and appear in the Appendix.

1. Observations - Animals were observed twice daily for toxicity and mortality.

Results - Toxicity - No dose related observed signs of toxicity were apparent during the observation period.

Mortality (Survival) - All dams survived to termination at gd 20.

2. Body Weight - They were weighed on gd 0, 6, 10, 14, 18, and 20. Carcass weights (body weight less uterus and contents) were also determined at gd 20.

Results - Body weights were statistically significantly less than control values on gd 10 (93%), 14 (89%), 18 (94%), and gd 10 (90%), 14 (86%), and 18 (91%) at the MDT and HDT; in addition, the carcass weight was decreased at the MDT (93%) and HDT (91%), respectively (Table 6.3). Body weight gains were statistically significantly less than control values between gd 6-10, and 10-14 at the MDT and HDT, and greater than control values between gd 14-18, 18-20 at the HDT (Table 6.4). Mean daily body weight gains in g are presented in Table A.

Mean Calculated Daily Weight Gain for the designated period in g.

	Group			
	1	2	3	4
Gestational period				
0-6	5.2	5.3	4.3	4.7
6-10	2.5	1.75	-1.75	-4.25
10-14	5.5	5.0	3.25	1.75
14-18	9.75	8.25	11.5	12.5
18-20	11.5	11.0	15.0	15.5

3. Food consumption and efficiency - Consumption was reported between gd 0-5, from gd 6 through gd 15, and on gd 16-17, and 18-19 (Table 6.2). Table B presents calculated daily food consumption during the comparable time periods of the calculated daily body weight gain in Table A. Relative efficiency of food utilization was not presented in the submitted report, but these values were calculated from the submitted data. The calculated values are presented in Table C. The following equation was used in these calculations:

Relative Efficiency = [mean daily body weight gain (kg)]/[mean daily food consumption (g)]

Results - Food consumption was statistically significantly decreased at the MDT and HDT during the dosing period from gd 6-15, and statistically significantly increased at these same dose levels after the dosing period, gd 15-19 (Table 6.2). Table C presents the results on the efficiency of food utilization. The relative efficiency of food utilization apparently decreased at the MDT and HDT for gd 7-10, and a nominal decrease occurred for gd 7-10 at the LDT and for gd 11-14 at the HDT. Rats gained the least body weight including controls, and consumed the least food during gd 6-10 (Table 6.4 and 6.2 of the Appendix).

Table B.

Mean Daily Food Consumption in g during the Designated Period.

	Group			
	1	2	3	4
Time period				
gd 0 thru 5	27.4	25.4	26.8	28.2
gd 7 thru 10	21.8	20.0	15.5	13.2
gd 11 thru 14	24.8	22.5	20.5	16.8
gd 15 thru 17	26.0	24.3	26.7	25.7
gd 18 thru 19	24.0	23.5	27.5	28.0

Table C.

Relative Efficiency of Food Utilization.

Group	1	2	3	4
Relative Efficiency for				
gd 0- 5	0.19	0.21	0.16	0.17
gd 7-10	0.11	0.09	-0.11	-0.32
gd 11-14	0.22	0.22	0.16	0.10
gd 15-17	0.38	0.34	0.43	0.49
gd 18-19	0.48	0.47	0.54	0.55

4. Necropsy of Dams and Fetal Examinations: Dams were sacrificed on gd 20 by CO_2 asphyxiation. The ovaries were removed and the corpora lutea counted. Uteri were removed and the number of dead fetuses counted, and viable fetuses counted and weighed; implantation sites were also counted. Reproduction data were reported in Table 6.5 of the Appendix. No dose related effects were noted in the reproductive data.

All the fetuses were examined externally, and about 1/3 of each litter were examined viscerally by the method of Monie, Kho, and Morgan, 1965, a sectioning technique, and about 2/3 of each litter were examined skeletally after being stained with Alizarin.

a. Gross pathology - One dam in the medium dose group demonstrated clotted blood in the uterine horn of an otherwise normal pregnancy. No dose related effects occurred on the reproductive parameters (Table 6.5).

b. Fetal Examination - There were no dose related effects on mean fetal weights (Table 6.6). Viable litter size, live and dead fetuses, and post implantation loss were not different from control values.

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There were no dose related visceral malformations or variations (Table 6.7, 6.8, and 6.9). Random variations occurring included short or absent renal papillae, dilated ureters, dilated trachea, and mottled livers.

Statistically significant dose and treatment related skeletal effects occurred in the mid and high dose groups (Tables 6.10 for fetuses, and 6.11 for litters). The parameters affected on a litter basis were presphenoid, at the HDT, and additional lumbar vertebra/centra at the MDT and the HDT. These parameters occurred in a dose related manner in fetuses, but were not reported to be statistically significant (Table 6.10). In addition, the total number of variations were nominally increased and appeared to be dose related in fetuses at all dose levels.

The parameters statistically significantly affected on a fetal basis at the MDT and HDT were: head incompletely ossified, teeth not ossified, centra/vertebrae not ossified and/or additional, rudimentary ribs, and sternebrae not ossified. Only sternebrae were statistically significantly elevated on a fetal basis at the LDT as well as at the MDT and HDT. In addition, on a fetal basis (Tables 6.10), these and 5 other parameters were nominally elevated at all dose levels, except for the vertebra/centra not ossified and/or additional, and the presence of rudimentary rib. On a litter basis 11 parameters were nominally elevated at all dose levels, one of which was litters with none ossified sternebrae.

One of the skeletal parameters referred to above, unossified sternebrae, demonstrated a statistically significant dose related increase at all dose levels for fetuses but not for litters. On further evaluation of individual unossified sternebrae, the apparent dose relationship at the LDT was removed (Table D.) below.

The total number of fetuses and litters with these skeletal and other variations were nominally elevated at all dose levels, but none were statistically significant, except at the HDT (Table 6.10).

Table D.

The incidence of unossified sternebrae: The approximate number of litters with unossified sternebrae.[a]

	Group			
	1	2	3	4
Sternebrae Not Ossified				
Number 1[b]	0	0	2	4
2[b]	1	1	12	7
3[b]	0	1	1	6
4[b]	1	1	2	6
5[b]	16	21	18	18
6[b]	13	18	21	17

[a] The number of litters have not been checked, therefore, they should be considered as only approximate.
[b] Litters with unossified sternebra at this number.

D. DISCUSSION:

Maternal toxicity was demonstrated by a statistically significant decrease in body weight and body weight gain at the MDT and HDT during the treatment period. Food consumption and relative efficiency of food utilization were depressed at these same dose levels, and time period. Maternal toxicity was less clear at the LDT where a statistically significant decrease occurred in food consumption on gd 6, 14, and 15, and, nominally, in relative efficiency of food utilization for gd 7-10 (Table 6.2 and Table C), and the body weight and body weight gain were nominally depressed, but not statistically significantly depressed. These values are on the borderline for indicating maternal toxicity, and are considered sufficiently close to the NOEL, that the 30 mg/kg/day dose level will be considered the NOEL for maternal toxicity.

Fetal toxicity was demonstrated at the two highest dose levels. Centra/vertebrae (additional) were statistically significantly increased in litters at the MDT and HDT. Several other skeletal parameters indicating dose related toxicity at the MDT and HDT were statistically significant on a fetal basis, but not on a litter basis, such as: head not completely ossified, teeth not ossified, centra/vertebrae not ossified, rudimentary rib, and sternebrae not ossified. These parameters were nominally elevated in litters.

Most of the parameters affected frequently occur in association with maternal toxicity, and some may disappear if the fetuses were followed after birth, however these effects are considered indications of developmental toxicity in these studies.

The statistically significant increase in fetuses but in litters for unossified sternebrae at all dose levels may not be real, especially at the LDT. The increase in unossified

sternebrae at the LDT resulted from a high level of none dose related incidence of unossified sternebrae 5, and 6 in all groups, and a low incidence in sternebrae 1, 2, 3, and 4. A higher incidence of unossified sternebrae 2, and 3 resulted in the dose relationship at the MDT and the HDT (Table D), and the apparent dose related response at the LDT seen in Table 6.10. For this study, the high incidence in historical controls, the failure of the effects to be statistically significant in litters, and the interaction of the incidence of these 6 sternebrae (see Table D), the unossified sternebrae of the LDT are not considered to be an effect.

Thus, the statistically significant effects seen in fetuses appear to be real at the MDT and the HDT only. The nominally increased incidence in litters with these effects add to the significance of these effects. In addition, the statistically significant effects seen in litters (Table 6.11) on the presphenoid, and on the centra/vertebrae (additional) are also considered to be significant effects.

In general, these conclusions are in agreement with submitted report that fetal toxicity was demonstrated at maternally toxic dose levels only.

E. ADDITIONAL INFORMATION REQUESTED:

1. What preparation, such pulverization of the test material, was conducted prior to suspension in the carboxymethylcellulose-water vehicle?

2. What were the approximate particle sizes, and distribution of particle sizes of the test material suspended in the vehicle?

3. The data on the analyses of samples of the dosing suspensions used must be submitted.

4. The purity of the test material was not specified, although it could be determined from a submitted 90-dog study using the same batch of test material.

5. The source of the test animals was not stated.

F. APPENDIX

SIMAZINE

Page_____ is not included in this copy.

Pages 100 through 110 are not included.

The material not included contains the following type of information:

____ Identity of product inert ingredients.

____ Identity of product impurities.

____ Description of the product manufacturing process.

____ Description of quality control procedures.

____ Identity of the source of product ingredients.

____ Sales or other commercial/financial information.

____ A draft product label.

____ The product confidential statement of formula.

____ Information about a pending registration action.

__X__ FIFRA registration data.

____ The document is a duplicate of page(s) _____.

____ The document is not responsive to the request.

The information not included is generally considered confidential by product registrants. If you have any questions, please contact the individual who prepared the response to your request.

83-4

07449

Reviewed By: Henry W. Spencer, Ph.D. 7/24/87
Review Section II, Toxicology Branch I - IRS (H7509C)
Secondary Reviewer: Marion P. Copley, D.V.M. 5/1/87
Review Section II, Toxicology Branch I - IRS (H7509C)

DATA EVALUATION REPORT

Study Type: Three-Generation Reproduction Study in Rats (83-4)

TOX Chem No.: 740

MRID Nos.: 00023365, 00080631

Test Material: Simazine 80W (2-chloro-4,6-bis-(ethylamine)-s-triazine 80%). Received August 16, 1963; Lot No. PL 1380

Sponsor: Geigy-Ciba Research Laboratories
Yonkers, New York

Testing Facility: Woodard Research Corporation
Herndon, VA 22070

Title of Report: Simazine: Three-Generation Reproduction Study in the Rat.

Author: Carter D. Johnston, Ph.D.

Report Issued: September 14, 1965

Conclusion:

Parental toxicity NOEL = less than 50 ppm in male and female parents due to reduced weight gain in F_{1b} and F_{2b} generations. The weight gains of males were also significantly reduced by approximately 11 percent in the F_0 generation during their premating period when compared to the controls.

There were several male animals in groups F_0, F_1, and F_2 of the study who appeared to be unable to produce young but they were not histologically evaluated.

There is a suggestion that the treated F_{3b} pups examined histologically may have altered livers but too few animals were examined to be able to completely evaluate this effect.

A reproductive NOEL therefore cannot be determined due to the lack of evalution of apparently sterile males.

Core Classification: Supplementary

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A. Materials:

1. Test Compound - Simazine 80W; Lot No. PL 1380 described as 80% purity.

2. Test Animals - Species: Albino rats; Strain: Not supplied but delivered from Charles River Breeding Laboratories, Inc. at approximately 23 days of age and acclimated in the laboratory for 1 week prior to study commencement.

B. Study Design:

1. Animal Assignment - The F_0 generation was started by assigning 20/sex in a control group and a test group at 100 ppm of simazine in the diet.

2. Housing was in individual cages in temperature controlled rooms with food and water ad libitum.

3. After exposure to the test compound for 74 days, the two sexes were allowed to mate for 10 days.

4. The F_{1a} generation and litters were examined for the number of live pups and the mean litter weights, number of stillborn, and physical condition of the test subjects.

5. At weaning, each member of the litter was recorded with the mean weight and number of survivors and physical condition of the test subjects. After observation, the pups were sacrificed and autopsied.

6. A second mating of the F_0 parents followed with remating as different pairs. The observations were carried out for the F_{1b} litters.

7. At weaning, representative pups were selected to serve as the next parents. All other pups were sacrificed and necropsied. The parents were also sacrificed.

8. The addition of a new group (F_{1b}) of test animals at 50 ppm was added to the F_{1b} parents fed either 0 or 100 ppm in the diet. However, only 10 males and 20 females were used in each of the three groups. One male was mated with each of two females in the test group.

9. After exposure for 31 days, mating occurred with one-half the females and 10 days later with the second 10 females.

10. The second litters (F_{2b}) were the parents for the succeeding groups.

11. Dietary exposure and mating procedures as well as examination of the litters produced were as the preceding generation (F_1b).

12. F_3a and F_3b litters were produced. However, at weaning, the F_3a litters were sacrificed and the F_3b litters were autopsied.

13. Organ weights of the liver, kidney and heart of two of each sex/litter at weaning were determined. These three tissues plus the spleen, adrenal, thyroid, gonad, and bone marrow were preserved. One animal/sex/litter was examined histologically. At least nine per sex in each dosage group were examined.

14. Statistical evaluation was not carried out in the study.

C. Test Diet:

- The test diet was not assayed for homogeneity nor for the test dosages actually present in the diet. The diets were made with either 50 or 100 ppm of active ingredient with an 80% W.P. (Simazine 80W).

D. Methods, Results and Discussion:

1. Parental Animal Observations - No clinical signs of toxicity were reported in daily observations. However, body weight gains were reduced in the F_0 generation males. Reduced gains were observed as early as the second week of exposure to 100 ppm simazine.

   A 9.5 percent reduction in gain was seen by week 14 when compared to controls. Females in the F_0 generation were not affected by reduced weight gain.

2. F_1b Males - By week 16, there was a 7.7 percent decrease in weight in males when compared to weights of controls and by 26 weeks a 15.6 percent reduction in weight gain was noted at 100 ppm. The 50 ppm dosed diet, however appeared to affect a greater change than the 100 ppm dosage. At 16 weeks an 18.7 and a 22.7 percent loss in weight was registered for the 50 and 100 ppm groups, respectively, when compared to controls.

3. Females - The females in the F_1b generation lost weight (about 7.5 percent) by week 8 of the study. Both 50 and 100 ppm dosages appeared to be about equally effective in producing the weight loss.

4. The F_2b generation in females exhibited weight gain losses of about 10 percent by week 11 of the study at both 50 and 100 ppm.

5. Males in the F_2b generation appeared to be more affected by the 50 ppm dosage than the 100 ppm diet. As much as a 10 percent weight loss occurred at 50 ppm by week 17 of the study.

6. The adult animals were not weighed during gestation or lactation.

E. Food Consumption:

- Food consumption was not computed for animals in the study.

F. Sacrifice and Pathology:

1. Necropsy results were sparsely reported but no lesions relating to dietary exposure were reported.

2. Organ weights were not obviously different from controls. However a dose-related increasing trend in absolute liver weights was noted in the F_3b weanling males. Relative (organ to body weight ratio) weights were essentially the same and do not suggest an effect from the dosages of simazine.

G. Histopathology:

1. Several adult animals, including 3 of 10 males in one group (100 ppm) that appeared to have been sterile in their mating efforts, were not evaluated histopathologically.

2. The pups of the F_3b generation were examined histologically. Only one per sex in each litter was examined. Toxicity to tissues was not obvious in treated animals when compared to controls of such small numbers of animals.

H. Reproductive Parameters:

- Treated pup mean weights (g/pup/litter) at birth did not vary significantly from controls in any generation.

g/Pup/Litter

	Controls		Simazine 100 ppm		Simazine 50 ppm	
	1st Litter	2nd Litter	1st Litter	2nd Litter	1st Litter	2nd Litter
F_0	5.8	6.2	6.1	5.9	---	---
F_2	6.6	5.8	6.3	5.7	6.2	5.9
F_3	6.3	6.5	6.1	6.3	6.0	6.1

The percent of young alive at weaning noted in the following table did not indicate significant toxicity to the young during lactation.

	Controls		100 ppm		Simazine 50 ppm	
	1st Litter	2nd Litter	1st Litter	2nd Litter	1st Litter	2nd Litter
F_0	60%	80%	67%	89%	---	---
F_2	87%	39%	33%	89%	87%	88%
F_3	83%	76%	71%	80%	81%	86%

Stillbirths were variable throughout the different litters and generations and did not indicate a chemically-related effect.

Conclusions:

Parental NOEL < 50 ppm, LEL = 50 ppm based on reduced weight gains by males in the premating periods.

Reproductive toxicity NOEL/LEL could not be determined based on lack of histologic evaluations in apparently sterile males in the F_1b generation. Up to 33 percent of the potential paternal stock at 100 ppm did not produce a pregnant female in two successive breeding sessions. The small sample size of F_3b pups examined, and the length of gestation was not determined. Pup and litter weights at 14 and 21 days were not determined. No rationale for dose selection was given. Too few adult males were used in the breeding program. The male and female parents were not examined histologically in any generation.

~~Attachment 5~~

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CONFIDENTIAL BUSINESS INFORMATION
DOES NOT CONTAIN
NATIONAL SECURITY INFORMATION (EO 12356)

007449

EPA: 68-02-4225
DYNAMAC No. 378-A
July 29, 1988

DATA EVALUATION RECORD

SIMAZINE

Salmonella/Mammalian Microsome Mutagenicity Assay

STUDY IDENTIFICATION: Lasinski, E. R., Kapeghian, J. C., and Green, J. D. Simazine technical Salmonella/mammalian-microsome mutagenicity assay (Ames assay). (Unpublished study No. 87038 prepared by CIBA-GEIGY Corp., Summit, NJ for CIBA-GEIGY Corp., Greensboro, NC; dated July 8, 1987.) MRID No. 406144-06.

APPROVED BY:

Robert J. Weir, Ph.D.
Acting Department Manager
Dynamac Corporation

Signature: Robert J. Weir

Date: July 29, 1988

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007449

1. CHEMICAL: Simazine.

2. TEST MATERIAL: Simazine technical was described as a powder; the purity was not reported.

3. STUDY/ACTION TYPE: Salmonella/mammalian microsome mutagenicity assay.

4. STUDY IDENTIFICATION: Lasinski, E. R., Kapeghian, J. C., and Green, J. D. Simazine technical Salmonella/mammalian-microsome mutagenicity assay (Ames assay). (Unpublished study No. 87038 prepared by CIBA-GEIGY Corp., Summit, NJ for CIBA-GEIGY Corp., Greensboro, NC; dated July 8, 1987.) MRID No. 406144-06.

5. REVIEWED BY:

Nancy E. McCarroll, B.S.
Principal Reviewer
Dynamac Corporation

Signature: Nancy E McCarroll
Date: 7-29-88

I. Cecil Felkner, Ph.D.
Independent Reviewer
Dynamac Corporation

Signature: I Cecil Felkner
Date: 7-29-88

6. APPROVED BY:

I. Cecil Felkner, Ph.D.
Genetic Toxicology Studies
Technical Quality Control
Dynamac Corporation

Signature: I Cecil Felkner
Date: 7-28-88

Henry Spencer, Ph.D.
EPA Reviewer

Signature: G. Kocialski for HS
Date: 8/8/88

Albin Kocialski, Ph.D.
EPA Section Head

Signature: G. Kocialski
Date: 8/8/88

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7. CONCLUSIONS:

A. Simazine technical was evaluated in two independent Salmonella/mammalian microsome assays at five doses ranging from 10 to 250 µg/plate. The highest dose both with and without S9 activation precipitated; no level was cytotoxic or mutagenic in any assay strain under any condition. We conclude, therefore, that simazine technical was assayed to the limit of solubility with no evidence of a mutagenic effect in this test system.

B. The study is acceptable.

Items 8 through 10--see footnote 1.

11. MATERIALS AND METHODS (PROTOCOLS):

A. Materials and Methods: (See Appendix A for details.)

1. Test Material: Simazine technical was described as a powder; the purity was not reported. The test material was dissolved in dimethylsulfoxide (DMSO); information furnished by the sponsor indicated that the test material was stable in DMSO at room temperature for at least 3 days (see Appendix B, Analytical Data, CBI p. 27). Similarly, analytical data provided by the sponsor showed that the highest (250 µg/mL) and lowest (10 µg/mL) assayed doses were within 10% of the target concentration.

2. Test Organisms: S. typhimurium strains TA1535, TA100, TA1538, TA98, and TA1537 were obtained from B. N. Ames, University of California. Permanent stock cultures of the indicator organisms were held frozen. Cultures used in the assay were generated from the frozen stocks by inoculating into Oxoid Media No. 2 and growing the cultures for 12 hours at 37°C. Cultures were checked for their genetic identity/integrity and adjusted to 10^9 cells/mL.

3. S9 Activation: The S9 fraction was obtained from Bionetics, Charleston, SC, and was derived from the livers of Sprague-Dawley rats treated with Aroclor 1254. The S9 contained 0.08 to 0.2 mL S9/mL.

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[1]Only items appropriate to this DER have been included.

4. Preliminary Cytotoxicity Assay: Seven concentrations of the test materials, the solvent control (DMSO), and the positive control (sodium azide at 3 µg/plate) were assayed in the preliminary cytotoxicity test in the absence of S9 activation with strain TA100. Treated cultures were plated in duplicate for total survivors and mutant colonies. No further details were reported.

5. Mutation Assay:

   a. Procedure: Five concentrations of the test material were assayed in the presence or absence of S9 activation. To individual tubes containing 2.5 mL of supplemented molten agar (0.05 mM L-histidine and 0.05 mM biotin), 0.1 mL of the appropriate test material dilution, tester strain, solvent, or positive controls were added. For the S9-activated assay, 0.5 mL of S9 was added. The contents of each tube were mixed, poured onto Vogel Bonner minimal agar plates, and incubated at 37°C in the dark for 48 hours. Each dose of the test material, solvent, and positive controls was evaluated in triplicate plates. Sterility controls of the test material and S9 mix were included. At the end of the incubation period, revertant colonies were scored, and their means and standard deviations were calculated.

   b. Positive Controls: Nonactivated mutagen controls were assayed; they included sodium azide (0.3 µg/plate with TA1535 and 3.0 µg/plate with TA100), daunomycin (2 µg/plate with TA1538 and TA98), and 9-aminoacridine (40 µg/plate with TA1537). The S9-activated positive controls were benzo-(α)-pyrene (3 µg/plate with TA1538, TA98, and TA100), B-naphthylamine (10 µg/plate with TA1535), and 3-methylcholanthrene (10 µg/plate with TA1537).

6. Evaluation Criteria: The assay was evaluated as follows. If the solvent control values for each strains were within the normal range and the sensitivity of the test system to detect a mutagenic response was demonstrated, the test material was considered mutagenic when it produced a positive and reproducible dose response over three concentration levels with the lowest increase equal to twice the solvent control value.

B. Protocol: A protocol was not provided.

12. REPORTED RESULTS:

A. Preliminary Cytotoxicity Assay: The nonactivated cytotoxicity assay was conducted with seven doses ranging from 1 to 2000 µg/plate. Compound precipitation occurred at 250, 500, 1000, and 2000 µg/plate; however, the test material was not cytotoxic at any dose. Based on these results, the study authors selected the lowest insoluble level as the highest dose for the mutation assay.

B. Mutation Assay: Simazine technical was evaluated in two nonactivated and S9-activated mutation assays at doses of 10, 25, 50, 100, and 250 µg/plate. Individual results were presented for each assay but means and standard deviations for both experiments were combined. Both the individual and combined results indicate that the test material was neither cytotoxic nor mutagenic (Table 1).

Compound precipitation was, however, reported at the highest assayed dose 250 µg/plate. Results (also presented in Table 1) show that all strains responded to the mutagenic action of the appropriate nonactivated and S9 activated positive controls.

13. STUDY AUTHORS' CONCLUSIONS/QUALITY ASSURANCE MEASURES:

A. The authors concluded, "Based on established evaluation criteria, Simazine Technical was not mutagenic at concentrations up to the solubility limits of 250 µg per plate."

B. A quality assurance statement was signed and dated June 26, 1987.

14. REVIEWERS' DISCUSSION AND INTERPRETATION OF STUDY RESULTS:

We assess that the study was properly conducted and that the study authors correctly interpreted the data. Simazine technical was clearly shown to be noncytotoxic and nonmutagenic when assayed to the limit of solubility. By contrast, all strains responded to the appropriate nonactivated or S9-activated mutagen control, which demonstrates that the test system had an adequate level of sensitivity.

Item 15--see footnote 1.

16. CBI APPENDIX: Appendix A, Materials and Methods, CBI pp. 10-14, and Appendix B, Analytical Data, CBI p. 27.

TABLE 1. Representative Combined Results from the Salmonella typhimurium Mutagenicity Assays with Simazine Technical

Substance	S9 Activation	Dose (µg/plate)	Revertants per Plate of Bacterial Tester Strain[a]				
			TA1535	TA1537	TA1538	TA98	TA100
Solvent Control							
Dimethylsulfoxide	-	—	13 ± 2	13 ± 1	15 ± 3	30 ± 5	99 ± 10
	+	—	12 ± 2	10 ± 3	21 ± 5	32 ± 5	94 ± 8
Positive Controls							
Sodium Azide	-	0.3	165 ± 23	—	—	—	—
	-	3.0	—	—	—	—	673 ± 90
Daunomycin	-	2.0	—	—	63 ± 16	884 ± 88	—
9-Aminoacridine	-	40.0	—	192 ± 57	—	—	—
Benzo-(a)-pyrene	+	3.0	—	—	112 ± 15	197 ± 42	660 ± 88
B-Naphthylamine	+	10.0	318 ± 34	—	—	—	—
3-Methylcholanthrene	+	10.0	—	38 ± 12	—	—	—
Test Material							
Simazine	-	250[b]	12 ± 4	13 ± 4	16 ± 4	29 ± 5	94 ± 11
	+		13 ± 4	10 ± 4	21 ± 6	27 ± 3	88 ± 10

[a] Means and standard deviations were determined from six plates per treatment group (combined results of two separate experiments).

[b] Highest assayed dose; slight compound precipitation was seen at this level. Results for lower concentrations (10, 25, 50, and 100 µg/plate/+ or -S9) were comparable to the corresponding control values.

APPENDIX A

Materials and Methods

SIMAZINE

Page_____ is not included in this copy.

Pages 123 through 129 are not included.

The material not included contains the following type of information:

____ Identity of product inert ingredients.

____ Identity of product impurities.

____ Description of the product manufacturing process.

____ Description of quality control procedures.

____ Identity of the source of product ingredients.

____ Sales or other commercial/financial information.

____ A draft product label.

____ The product confidential statement of formula.

____ Information about a pending registration action.

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NATIONAL SECURITY [illegible] ON EO 12065)

EPA: 68-02-4225
DYNAMAC No. 378-C
August 3, 1988

DATA EVALUATION RECORD

SIMAZINE

Mutagenicity--Unscheduled DNA Repair in
Primary Rat Hepatocytes

STUDY IDENTIFICATION: Puri, E. Autoradiographic DNA repair test on rat hepatocytes. (Unpublished study No. 830640 prepared by CIBA-GEIGY Ltd., Basle, Switzerland, for CIBA-GEIGY Corp., Greensboro, NC; dated December 20, 1983.) MRID No. 406144-08.

APPROVED BY:

Robert J. Weir, Ph.D.
Acting Department Manager
Dynamac Corporation

Signature: Robert J. Weir

Date: 8/3/88

1. CHEMICAL: Simazine; G 27 692.

2. TEST MATERIAL: G 27 692 was from lot No. 209158 and had a purity of 99.6%.

3. STUDY/ACTION TYPE: Mutagenicity--Unscheduled DNA repair in primary rat hepatocytes.

4. STUDY IDENTIFICATION: Puri, E. Autoradiographic DNA repair test on rat hepatocytes. (Unpublished study No. 830640 prepared by CIBA-GEIGY Ltd., Basle, Switzerland, for CIBA-GEIGY Corp., Greensboro, NC; dated December 20, 1983.) MRID No. 406144-08.

5. REVIEWED BY:

Nancy E. McCarroll, B.S.
Principal Reviewer
Dynamac Corporation

Signature: Nancy E. McCarroll
Date: 8-3-88

I. Cecil Felkner, Ph.D.
Independent Reviewer
Dynamac Corporation

Signature: Sharon Andrae for
Date: 8-3-88

6. APPROVED BY:

I. Cecil Felkner, Ph.D.
Genetic Toxicology Studies
Technical Quality Control
Dynamac Corporation

Signature: Sharon Andrae for
Date: 8-3-88

Henry Spencer, Ph.D.
EPA Reviewer

Signature: Henry Spencer
Date: 9/12/88

Albin Kocialski, Ph.D.
EPA Section Head

Signature: A. Kocialski
Date: 9/12/88

7. CONCLUSIONS:

A. Under the conditions of this unscheduled DNA synthesis (UDS) assay, the reevaluation of code slides prepared from primary rat hepatocyte cultures exposed to 0.4, 2, 10, and 50 µg/mL G 27 692 for 5 hours did not show a significant increase in net nuclear grain counts. However, the length of exposure may have been too short to provide optimal conditions for the detection of UDS induction by the test material (see Reviewers' Discussion and Interpretation of Study Results, Section 14). Additionally, the author stated that the high dose was selected based on the solubility properties of the test material; however, no data were presented to support this statement.

B. The study is unacceptable.

8. RECOMMENDATIONS:

The assay should be repeated using the recommended 18-hour exposure time [1] and the author should provide sufficient data to assure that the highest dose assayed is not soluble.

Items 9 and 10--see footnote 2.

11. MATERIALS AND METHODS (PROTOCOLS):

A. Materials and Methods: (See Appendix A for details.)

1. Test Material: G 27 692 from lot No. 209158 was listed as 99.6% pure. No information on physical appearance, stability, or storage conditions was provided. The test material was soluble in dimethylsulfoxide (DMSO) at 5 mg/mL.

2. Indicator Cells: Primary rat hepatocytes were collected by in situ collagenase perfusion of the liver of male Tif RAIf (SPF) rats (170-200 g) obtained from CIBA-GEIGY Tierfarm, Sisseln.

3. Cell Preparation:

a. Perfusion Technique: The liver was perfused for 8 minutes with a balanced salt solution (BSS) containing 5 mM glucose, pH 7.4, and with 0.5% collagenase-supplemented

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[1] Mitchell, A. D., Casciano, D. A., Meltz, M. L., Robinson, D. E., San, R. H. C., William, G. M., and Von Halle, E. S. Unscheduled DNA synthesis tests, a report of the U.S. Environmental Protection Agency Gene-Tox Program. Mutat. Res. 123(1983):363-410.

[2] Only items appropriate to this DER have been included.

BSS for 15-20 minutes. The liver was excised, removed to a culture dish containing Hanks' solution, and shaken to release the hepatocytes.

b. Hepatocyte Harvest/Culture Preparation: Recovered cells were filtered, suspended in Williams' Medium E (WME), counted, and dispensed ($3x10^5$cells) onto gelatinized coverslips in multi-well culture plates. The cultures were placed in a humidified, 37°C, 5% CO_2 incubator for a 1.5- to 2-hour attachment period. Unattached cells were removed; viable cells were refed and established as monolayer cultures.

4. Preparation Cytotoxicity Assay: Cells initiated from the primary culture were exposed to seven concentrations of the test material and the solvent control for 5 hours. Dosed cells were rinsed, stained with Trypan blue, and fixed, and the percentage of unstained cells in 100 scored hepatocytes was determined. The following criteria were used to evaluate the cytotoxicity results and to establish doses for the UDS assay: a sufficiently large number of cells must adhere to the coverslip, at least 25% of the cells must show viability upon examination by means of the vital-staining techniques, and a corresponding percentage of the cells must be in good condition upon morphological examination.

5. UDS Assay:

a. Treatment: Four preselected concentrations of the test material were evaluated in the UDS assay. Triplicate cultures per group were exposed to the test material doses, the negative control (untreated), the solvent control (DMSO), and the positive control (100 mM dimethylnitrosamine, DMN) in the presence of 1 µCi/µL [^{3}H]thymidine for 5 hours. Exposed cells were washed and fixed with ethanol/acetic acid (3:1) and the coverslips were mounted onto slides.

b. Preparation of Autoradiographs/Grain Development: Slides were coated with Kodak AR10, dried for 6 days at 4°C in light-proof dessicated boxes, developed in Kodak D-19, fixed, stained in hematoxylin and eosin, coded, and counted.

c. Grain Counting: Nuclear grains of 150 cells for each treatment group were counted. Net nuclear grain counts were determined by subtracting the nuclear grain counts of each cell from the mean cytoplasmic grain counts.

6. Evaluation Criteria:

a. Assay Validity: The assay was considered valid if hepatocyte viability prior to treatment was >70%; gross nuclear grain counts in the solvent control did not exceed 8 total grains/nucleus or the percent of solvent

control nuclei with >5 grains/nucleus did not exceed 10%; the positive control fulfilled all criteria for a positive response; and grain counts for a given treatment were obtained from at least two replicate cultures.

b. Positive Response: The test material was reported as positive if the mean gross nuclear grain count was >2-fold higher than the solvent control at any dose or if a dose-related increase in the mean gross nuclear grain count, with at least one concentration showing a significant increase over the solvent control, was achieved.

7. Statistical Methods: The gross nuclear grain counts were analyzed by Duncan's multiple range test at $p \leq 0.01$.

8. Protocol: A protocol was not provided; however, primary data from the slide analysis, historical background data, and summarized findings from an initial slide evaluation were furnished.

12. REPORTED RESULTS:

A. Preliminary Cytotoxicity Assay: The cytotoxicity assay was performed with seven test concentrations ranging from 0.78 to 50 µg/mL. The author stated that 50 µg/mL was selected as the high dose based on the solubility properties of the test material. The test material did not cause any appreciable cytotoxic response at any assayed dose; therefore, the high dose for the UDS assay was selected on the basis of compound insolubility.

B. UDS Assay: The four doses selected for the UDS assay were 0.4, 2, 10, and 50 µg/mL. The author stated that the original assay, conducted in November 1983, was performed with uncoded slides and results were presented as gross nuclear grain counts. To correct these deficiencies, slides were coded and reevaluated in March 1988. As shown in Table 1, the results from the reevaluated slides indicated that the test material did not cause an appreciable increase in net nuclear grain counts at any of the four assayed doses. By contrast, exposure of the hepatocytes to 100 mM DMN caused an increase in UDS. The test material results confirmed the findings from the initial slide analysis, which indicated that G 27 692 was negative in this test system.

13. STUDY AUTHOR'S CONCLUSIONS/QUALITY ASSURANCE MEASURES:

A. The author stated: "It is concluded that, under the given experimental conditions, no evidence of induction of DNA damage by G 27 692 or by its metabolites was obtained that could be interpreted as suggestive of mutagenic or carcinogenic properties of the substance."

TABLE 1. Representative Results of the Unscheduled DNA Synthesis Rat Hepatocyte Assay with G 27 692 (Reevaluated Slides)

Treatment	Dose/mL	No. Cells Scored	Mean[a] Nuclear Grain Count ± SD	Mean[a] Cytoplasmic Grain Count ± SD	Mean[a] Net Nuclear Grain Count ± SD
Negative Control	--	150	2.13±1.32	1.91 ± 0.97	0.22 ± 1.48
Solvent Control Dimethylsulfoxide	--	150	2.13 ± 1.31	2.17 ± 1.12	-0.04 ± 1.67
Positive Control Dimethylnitrosamine	100 mM	150	13.63 ± 3.72[b]	4.45 ± 1.74	9.17 ± 3.96
Test Material G 27 692	50 µg[c]	150	3.28 ± 1.85	2.95 ± 1.56	0.33 ± 2.06

[a]Triplicate cultures.

[b]Fulfills reporting laboratory's criteria for positive effect (mean nuclear grain count must be >2-fold higher than the solvent control value).

[c]Highest assayed dose was reported to be at the limit of test material solubility; results for lower doses (0.4, 2, and 10 µg/mL) were comparable to the solvent control value.

B. Signed quality assurance statements for the initial assay dated December 16, 1983, and for the reevaluation of the slides dated May 5, 1988, were present.

14. REVIEWERS' DISCUSSION AND INTERPRETATION OF STUDY RESULTS:

We assess that since the author stated that solubility limited the evaluation of higher test material concentrations, data should have been provided which indicated that 50 µg/mL was a precipitating dose. Additionally, the length of exposure of the hepatocytes to the test material (5 hours) may have been too short to detect a UDS response. The U.S. Environmental Protection Agency Gene-Tox Program[3] recommends an 18-hour exposure.

Although the ability of the hepatocytes to detect UDS induced by DMN following the 5-hour exposure was clearly demonstrated, the test material (G 27 692, simazine; 2-chloro-4,6 bis (ethylamino)-s-triazine) is not structurally related to DMN. Therefore, showing assay sensitivity to detect a known genotoxic agent after a short exposure provides no assurances that the conditions were optimal for the test material to interact with and damage genetic material. To illustrate the point, Barfknecht et al.[4] have shown that while DMN-induced UDS in rat hepatocytes is detected following a 4-hour exposure, increasing the exposure time to 18 hours markedly improved assay sensitivity relative to the magnitude of the response. Furthermore, the detection of activity spanned a wider range of DMN doses.

The issues relating to assay sensitivity and specificity were previously discussed with the sponsor (see summary of EPA/CIBA-GEIGY meeting conducted by Dr. Jane Harris, EPA Section Head, Toxicology Branch, on June 20, 1986). The consensus opinion of participants at this meeting was that the exposure time for the UDS assay should be 15-18 hours.

We conclude, therefore, that the assay should be repeated to conform with recommended procedures.

Item 15--see footnote 2.

16. CBI APPENDIX: Appendix A, Material and Methods, CBI pp. 9-11 and 20-22.

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[3]Mitchell et al. Mutat. Res. 123(1983):363-410.

[4]Barfknecht, T. R., Naismith, R. W. and Kornburst, D. J. Variations on the standard protocol design of the hepatocyte DNA repair assay (manuscript submitted to the J. Appl. Toxicol.).

APPENDIX A

Materials and Methods

SIMAZINE

Page_____ is not included in this copy.

Pages 138 through 143 are not included.

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____ Description of quality control procedures.

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____ The product confidential statement of formula.

____ Information about a pending registration action.

X FIFRA registration data.

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CONFIDENTIAL BUSINESS INFORMATION
DOES NOT CONTAIN
NATIONAL SECURITY INFORMATION (EO 12065)

EPA: 68-02-4225
DYNAMAC No. 378-8
August 3, 1988

DATA EVALUATION RECORD

SIMAZINE

Mutagenicity--In vitro Cytogenetic Study with Human Lymphocytes

STUDY IDENTIFICATION: Dollenmeier, P. Structural chromosomal aberration test--Chromosome studies on human lymphocytes in vitro. (Unpublished study No. 871099 prepared by CIBA-GEIGY Ltd., Basle, Switzerland, for CIBA-GEIGY Corp., Greensboro, NC; dated March 24, 1988.) MRID No. 406144-07.

APPROVED BY:

Robert J. Weir, Ph.D.
Acting Department Manager
Dynamac Corporation

Signature: [signature]

Date: 8/3/88

1. CHEMICAL: Simazine; G 27 692.

2. TEST MATERIAL: G 27 692 technical from lot No. 209 158 was listed as 99.6% pure.

3. STUDY/ACTION TYPE: Mutagenicity--In vitro cytogenetic study with human lymphocytes.

4. STUDY IDENTIFICATION: Dollenmeier, P. Structural chromosomal aberration test--Chromosome studies on human lymphocytes in vitro. (Unpublished study No. 871099 prepared by CIBA-GEIGY Ltd., Basle, Switzerland, for CIBA-GEIGY Corp., Greensboro, NC; dated March 24, 1988.) MRID No. 406144-07.

5. REVIEWED BY:

Nancy E. McCarroll, B.S.
Principal Reviewer
Dynamac Corporation

Signature: Nancy E McCarroll
Date: 8-3-88

I. Cecil Felkner, Ph.D.
Independent Reviewer
Dynamac Corporation

Signature: Sharon Andrews for
Date: 8-3-88

6. APPROVED BY:

I. Cecil Felkner, Ph.D.
Genetic Toxicology Studies
Technical Quality Control
Dynamac Corporation

Signature: Sharon Andrews for
Date: 8-3-88

Henry Spencer, Ph.D.
EPA Reviewer

Signature: Henry B. Spencer
Date: 9-9-88

Albin Kocialski, Ph.D.
EPA Section Head

Signature: A. Kocialski
Date: 9/9/88

7. CONCLUSIONS:

A. Under the conditions of the nonactivated and S9-activated human lymphocyte cytogenetic assay, five concentrations (6.25, 12.5, 25, 50, and 100 µg/mL) of G 27 692 (simazine) were neither cytotoxic nor clastogenic. However, several factors preclude acceptance of these results as valid evidence of a negative response:

1. The author stated that the highest dose was selected based on the solubility properties of the test material; however, no data were presented to support this statement.

2. Post treatment cell harvest was at 43.5 hours. To ensure that first division metaphases were available for analysis of compound-related effects, the cell harvest should have been performed at 24 hours.

B. The study is unacceptable.

8. RECOMMENDATIONS:

It is recommended that the assay be repeated using the appropriate cell harvest time and that either separate experiments with lymphocytes from different donors or replicate cultures from different donors be included. Additionally, the author should furnish data which indicates that the highest dose assayed is not soluble.

Items 9 and 10--see footnote 1.

11. MATERIALS AND METHODS (PROTOCOLS):

A. Materials and Methods: (See Appendix A for details.)

1. G 27 692 technical (simazine technical) from lot No. 209 158 was listed as 99.6% pure. No information on the physical appearance, storage conditions, or other characteristics that define the test material were reported. The test material was dissolved in dimethylsulfoxide (DMSO) and filter sterilized through a 0.2-µm membrane. Based on the solubility properties of the test substance, stock solutions for all assays contained 10.0 mg/mL; subsequent dilutions were prepared in DMSO and were added to the cell suspension to yield 1:100 dilutions. Solutions of 625 µg/mL (date of preparation was not reported) were analyzed for test material concentration and found to contain between 95 and 111% of the target concentration (see Appendix 3, Analytical Data, CBI pp. 18-21.)

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[1]Only items appropriate to this DER have been included.

2. Cell Line: Human lymphocytes were obtained from the venous blood of a single healthy donor; no information regarding the donor was provided. Cultures were grown in "conventional blood culture medium" (Chromosome Medium, Gibco) for 46 hours. The mitogen used to stimulate the lymphocytes was not specified; however, we assume that the Gibco product contained phytohemagglutinin.

3. S9 Fraction: The S9 fraction was obtained from Analabs Inc., North Haven, CT, and was derived from the livers of male RAI rats induced with Aroclor 1254. The S9 reaction mixture contained 0.15 mL S9 fraction.

4. Preliminary Cytotoxicity Assay: Cultures of lymphocytes were exposed to 14 concentrations of the test material or the solvent (DMSO) for 3 hours, both in the presence and absence of S9 activation. Treated cells were washed and reincubated in fresh medium for 24 hours. The mitotic index was determined by counting at least 1000 cells per dose group and the dose that induced an ~50% reduction in mitotic activity was selected as the highest concentration for the cytogenetic assay.

5. Cytogenetic Assay:

   a. Treatment: Duplicate cultures were exposed to five selected nonactivated and S9-activated concentrations of the test material, the solvent (DMSO), or the positive controls (0.8 µg/mL mitomycin C/-S9 or 10.0 µg/mL cyclophosphamide/+S9) for 3 hours. Following treatment, cells were washed, resuspended in fresh medium, and incubated for 43.5 hours. Colcemid (0.4 µg/mL) was added 2.5 hours prior to harvest. Metaphase cells were collected, swollen with a hypotonic 0.075 M KCl, and fixed in methanol:acetic acid (3:1). Slides were prepared and coded; the staining methods were not reported.

   b. Metaphase Analysis: One hundred cells from each treatment group (50 cells/culture) were examined for chromosome aberrations.

6. Statistical Methods: The data were not evaluated statistically.

7. Evaluation Criteria: No criteria to establish the validity of the assay or the biological significance of the results were provided.

8. Protocol: A protocol was not presented; however, a Standard Operating Procedure (SOP No. 301502) was listed.

12. REPORTED RESULTS:

A. Preliminary Cytotoxicity Assay: The cytotoxicity assay was conducted with 14 test doses ranging from 0.012 to 100 µg/mL, both in the presence and absence of S9 activation. No appreciable cytotoxicity or mitotic suppression was noted at any dose level. The study author stated that the highest concentration, 100 µg/mL used for the cytogenetic assays was based on the solubility of the test material in DMSO; solubility in culture medium was not reported.

B. Cytogenetic Assay: The five doses for the nonactivated and S9 activated assay were 6.25, 12.5, 25, 50, and 100 µg/mL. As shown in Table 1, evaluation of metaphases from lymphocyte cultures exposed to the five selected test material doses did not reveal an appreciable increase in the percentage of cells with chromosome aberrations. By contrast, exposure to both the nonactivated positive control, 0.8 µg/mL mitomycin C, and the S9-activated positive control, 10 µg/mL cyclophosphamide, induced marked increases in the percentage of aberrant cells.

13. STUDY AUTHOR'S CONCLUSIONS/QUALITY ASSURANCE MEASURES:

A. The study author stated, "It is concluded that under the given experimental conditions no evidence of mutagenic effects was obtained on human lymphocytes *in vitro* treated with G 27 692 tech."

B. A quality assurance statement was signed and dated March 22, 1988.

14. REVIEWERS' DISCUSSION AND INTERPRETATION OF STUDY RESULTS:

We assess that Simazine technical was not adequately tested for the potential to induce chromosome aberrations in human lymphocytes for the following reasons:

1. The author provided no data to support the statement that the highest assayed dose was based on test material insolubility.

2. Posttreatment cells were harvested at 43.3 hours. Since the maximum yield of first division metaphase would occur at 24 hours postexposure, preparations from cells cultured for longer periods will contain increasing proportions of cells in second and subsequent divisions. The data did not indicate that simazine technical caused mitotic delay; therefore, the use of a prolonged recovery time reduces the sensitivity of the test system to detect weak clastogenic activity.

Although not required, it is strongly recommended, however, that *in vitro* human lymphocyte cytogenetic assays should be performed with lymphocytes collected from different donors (i.e., each culture at each experimental point should be from separate donors or the entire experiment should be repeated with new donor lymphocytes).

Item 15--see footnote 1.

16. CBI APPENDIX: Appendix A, Materials and Methods, CBI pp. 10-12, and Appendix B, Analytical Results, CBI pp. 18-21.

TABLE 1. Representative Results of the Human Lymphocyte *In Vitro* Cytogenetic Assay with G 27 692 Technical

Substance	Dose (µg/mL)	S9 Activation	No. of Cells Scored	Total No. of Aberrations	% Cells with Aberrations
Solvent Control					
Dimethylsulfoxide	--	-	100	3	3
	--	+	100	2	2
Positive Control					
Mitomycin C	0.8	-	100	34	34[a]
Cyclophosphamide	10.0	+	100	18	13[a,b]
Test Material					
G 27 692	100[c]	-	100	1	1
	100	+	100	1	1

[a] Reported as positive by the study author.

[b] Report lists 13% of the scored metaphases with aberrations; since the total number of aberrations and % cells with aberrations do not agree, we assume that several cells had >1 aberration.

[c] Highest assayed dose was not cytotoxic but was reported by the author to be the limit of test material solubility in dimethylsulfoxide (i.e. 10,000 µg/mL); solubility in culture medium was not reported. Results for lower doses (6.25, 12.5, 25, and 50 µg/mL) were comparable to the solvent control value.

APPENDIX A

Materials and Methods

SIMAZINE

Page_____ is not included in this copy.

Pages 152 through 159 are not included.

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85-3

007449

Reviewed by Robert P. Zendzian Ph.D.
Secondary Reviewer: -
Date: August 24, 1988

See Memorandum: Simazine, Review of Dermal Absorption Study
from Robert P. Zendzian, Ph.D.
to Mike Ioannou, Ph.D.
Dated: August 24, 1988

Reevaluated by: Henry W. Spencer, Ph.D. 6/30/89
Secondary Reviewer: Marion P. Copely, DVM 6/30/89

Data Evaluation Report

Chemical : Simazine (14-C)

Toxicity Chemical No. 740

Purity: Simazine Technical, 96 to 98 % radio purity

Study Type: Dermal Absorption in rats

MRID No. 406144-09

Acc. No. -

Sponsor: Ciba-Geigy Corp.

Testing Facility: WIL Research Labs;;and Agrisearch Inc.

Title of Report: Dermal Absorption of 14C-Simazine in the rat

Authors: T. Murphy and G. Orr

Study No. ABR-88042

Report Issued: March 30, 1988

Conclusion:

The previous reviewers evaluation (copy attached) accurately reflects the results of the study. The study is classified as acceptable.

The reviewer found that at doses of 1 and 5 mg/rat (0.1 and 0.5 mg/cm2) and exposures of 2, 4, 10 and 24 hrs, actual mean dermal absorption was less than one percent. However, between 11 and 20% of the low dose and 31 and 41 % of the high dose remained on the skin after soap and water wash. This quantity is potentially absorbable.

An additional dermal absorption study was recommended by the reviewer to better quantitate the risk associated with dermal exposure.

85-3

007449

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

OFFICE OF
PESTICIDES AND TOXIC SUBSTANCES

MEMORANDUM August 24, 1988

SUBJECT: Simazine, Review of Dermal Absorption Study

TO: Mike Ioannou Ph.D.
Toxicologist

FROM: Robert P. Zendzian PhD
Senior Pharmacologist

Action Requested

Review the following dermal absorption study:

Dermal absorption of ^{14}C-Simazine in the rat, T. Murphy and G. Orr, CIBA-GEIGY Corporation, Ag Div, Biochemisty Dept.; WIL Research Labs; and Agrisearch Incorporated. Laboratory/Study No. ABR-88042, Mar 30, 1988, MRID 406144-09.

Conclusion

The study is acceptable.

At doses of 1 and 5 mg/rat (0.1 & 0.5 mg/cm2) and exposures of 2, 4, 10 and 24 hours, actual mean dermal absorption was less than one percent. However, between 11 and 20 % of the low dose and 31 and 41 % of the high dose remained on/in the skin after soap and water wash. This quantity is potentially absorbable.

Recommendation

An oncogenic risk, mammary gland tumors in female rats, has been identified for this compound. In order to better quantitate the risk associated with dermal exposure to this compound, an additional dermal absorption study is recommended. This study is designed to determine the fate of the material remaining on/in the skin following the soap and water wash.

Groups of twenty rats each should be treated dermally with 0.1 or 0.5 mg/cm2 simazine. Ten hours after dosing the application site should be washed, quantitatively, with soap and water. Four animals per dose should be terminated at this time. At intervals of 1, 2, 7 and 14 days after the wash, four animals per dose should be terminated. Methodology and sample collection should be as performed in this study.

Attachments
DER
One-liner

Data Evaluation Report 007449

Compound tested Simazine

Citation
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Reviewed by Robert P. Zendzian Ph.D.
Senior Pharmacologist

Core Classification Acceptable

Conclusions
At doses of 1 and 5 mg/rat (0.1 & 0.5 mg/cm^2) and exposures of 2, 4, 10 and 24 hours, actual dermal absorption was less than one percent. However, between 11 and 20 % of the low dose and 31 and 41 % of the high dose remained on/in the skin after soap and water wash. This quantity is potentially absorbable.

Materials

Simazine, ^{14}C-labeled in the triazine ring.
28.0 uCi/mg for the low dose 98% radio pure
2.4 uCi/mg for the high dose 96% radio pure

Charles Rivers Sprague-Dawley male rats 200-300 gms from Madison Wis.

Experimental design

"^{14}C-Simazine was dermally applied at two dosage levels of either 1.0 or 5.0 mg/rat. Four male rats were treated per time point and sacrificed at either 2, 4, 10 or 24 hours after treatment." The high dose was administered in a 50 uL aqueous suspension and the high dose in a 200 uL suspension. "The dorsal hair of all rats utilized in this study was shaved approximately 20-24 hours prior to dosing and the area washed with acetone. A 10 square-centimeter area, 4.0 cm by 2.5 cm" was used as the dosing area. The dose was uniformly spread with a Drummond displacement pipette. The amount of radio active simazine remaining in the pipette was determined. the dose was allowed to air dry and the dosing area was covered with a protective appliance.

Treated animals were housed separately in nalgene metabolism cages for the duration of the exposure. At termination the animals were anesthetized and the protective appliance removed for analysis. The application site was washed in situ with Dove liquid and water and rinsed with water. After washing the skin of the dosed area and the surrounding skin covered by the protective device were collected separately.

The carcass and total urine and feces were collected for analysis.

"The following samples were analyzed at the time of sacrifice; skin I (treated area), skin II (area covered by the application), soap rinse, water rinse, paper rinse, stomahesive rinse, bridge rinse, urine, feces, cage wash, paper, gauze squares (A and B), blood and carcass."

Results and Discussion

Results are summarized in tables II, III and IV from the report. The report includes material remaining on/in the skin after washing as absorbed. It is considered better to distinguish between material 'actually' absorbed (in blood, carcass, urine and feces) and material 'potentially' absorbable (on/in the skin). Less than one percent of the applied dose was actually absorbed even after 24 hours exposure. However, significantly high portions of the dose remained on the skin following the soap and water wash and were potentially available for absorption.

Very little evidence of time dependency for absorption and skin binding was observed with either dose. This is esssentially a function of the amount of materal in the carcass. Excretion showed a clear increase with time.

TABLE II: THE PERCENT OF DOSE ABSORBED[1], UNABSORBED[2], AND REMAINING ON THE SKIN AFTER A SOAP AND WATER RINSE IN ANIMALS TREATED WITH ^{14}C-SIMAZINE AT THE LOW DOSE LEVEL[3]

Fraction	Dose (1.0 mg/Rat) Time of Sacrifice (Hours) 2	4	10
Blood	0.00	0.01	0.00
Carcass	0.14	0.50	0.20
Urine	0.02	0.04	0.10
Feces	0.00	0.00	0.00
	0.16	0.55	0.30 not fully absorbed
Skin I	11.74	10.15	16.92
Skin II	1.49	1.33	1.50
Σ Skin	13.23	11.48	18.42 not fully absorbed
Absorbed	13.39	12.03	18.72
Bandage Rinse	0.06	0.03	0.39
Bridge Rinse	0.00	0.00	0.00
Paper Rinse	0.01	0.18	0.02
Soap Rinse	84.13	85.80	74.65
Water Rinse	4.72	4.47	6.97
Paper	0.00	0.00	0.00
Gauze A	5.52	5.62	4.84
Gauze B	0.16	0.14	0.23
Cage Wash	0.00	0.02	0.02
Unabsorbed	94.60	96.26	87.12
Total ^{14}C Recovered	107.99	108.29	105.84

[1]Sum of the blood, carcass, urine, feces, skin I, and skin II.

[2]Sum of the bandage rinse, bridge rinse, paper rinse, soap rinse, water rinse, paper, gauze A, gauze B, and cage wash.

[3]Mean of four animals per data point.