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

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JUL 31 1989

MEMORANDUM

OFFICE OF
PESTICIDES AND TOXIC SUBSTANCES

SUBJECT: Simazine, Toxicology Chapter of the Registration Standard (Second Round Review) Tox. Chem. No. 740
Cas No. 122-34-9

TO: Lois Possi, Acting Chief
Reregistration Branch
Special Review and Reregistration Division (H7508C)

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Attached is the Toxicology Chapter of the Final Registration Standard and the Tolerance Reassessment (FRSTR) for Simazine. The following portions are available in Lexitron disc.

A. Toxicology Summary
B. Toxicology Profile
C. Data Gaps
D. ADI (PfD) Reassessment
E. Toxicological Issues
F. Toxiclogy Summary Tables
G. Bibliography

This package includes only Data Evaluation Reports that support this Standard (FRSTR). Some of these Data Reports were included in the previous Registration Standard (RS-84).

Additional copies of the completed science chapter will be distributed as follows:

Robert Coberly, SACB/HED (H7509C)
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Toxicology Chapter
of the
Simazine
Second Round Review

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SIMAZINE

A. Toxicology Summary

Simazine is one of the class of s-triazine compounds which are herbicides. It is a selective herbicide used for the control of the majority of the annual grasses and broadleaf weeds in a variety of crops including corn, cherries, peaches, grapes, apples, and sod grass production, to list a few. Simazine when used at higher rates, becomes a nonselective herbicide for weed control in industrial areas.

Simazine technical is a fairly nontoxic (oral Toxicity Category IV) chemical with an oral LD_{50} of greater than 5 g/kg in rats. Dermally, the chemical is in Toxicity Category III with the dermal LD_{50} in rabbits of greater than 2 g/kg bwt.

There was minor toxicity following a 4 hour exposure to Simazine dust via the inhalation route with nominal exposures of 14.7 mg/L(actual exposure concentration of 1.71 mg/L). The toxicity category was III and the LC_{50} > 1.71 mg/L.

Technical Simazine was only slightly irritating following a 4-hour dermal exposure in rabbits with a primary irritation score (PIS) of 0.2 which placed the chemical in Toxicity Category IV.

Technical Simazine, when tested for eye irritation potential in rabbits produced a slight redness within 1 hour which totally resolved by 24 hours. The technical product is in Toxicity Category IV as a minimal irritant to the eyes. However, Simazine 80W produced moderate irritation by 72 hours which resolved by day 7 of observation. The 80W formulation is a Toxicity Category III chemical.

Dermal sensitization studies, using albino male guinea pigs challenged with technical Simazine, indicated no evidence for sensitization at the application sites.

Subchronic oral exposure to Sprague-Dawley rats for 13 weeks to Simazine in the diet caused reductions in weight gain and a reduced production of red blood cells.

Following a 13 week oral exposure, dogs had the same type of weight gain depression noted in the 13week rat study. The exposure in the diet was also associated with a reduced serum albumin and increased globulins in male dogs after 90 days of exposure.

A 21-day subacute/subchronic dermal toxicity study in rabbits indicated that toxic effects were absent from exposures at up to 1000 mg/kg/day (HDT).

No data are available on the subchronic inhalation toxicity of Simazine.

A recent 2-year rat study using Simazine in the diet caused significant reductions in hematologic parameters (RBC,

Hgb, Hct) and reduced body weight gains. A NOEL for systemic toxicity was determined to be 10 ppm (0.52 mg/kg/day in females) with an LEL = 100 ppm (5.34 mg/kg bwt/day in females). Increased incidences of mammary tumors were seen in female rats when compared to historical and concurrent controls.

A 2-year chronic feeding study in the mouse using Simazine in the feed studied 60 animals per sex at each dosage. The loss of body weight gain and reduction in hematologic parameters are seen at 1000 ppm (approximately 143 mg/kg/day). These are consistent with effects seen in other species. A NOEL for these effects was established at 40 ppm (approximately 4.7 mg/kg/day). The LEL was 1000 ppm (142 mg/kg/day). No tumorigenic response was noted following the ingestion of Simazine for 2 years.

The oncogenic potential of simazine was recently reviewed by the Health Effects Division Peer Review Committee. The Committee concluded from the data submitted for review that the weight of the evidence supported a classification of C oncogen with a Q_1^* quantitative risk evaluation of the chemical.

A 1-year study in dogs fed simazine in the diet showed losses in body weight gain at the HDT (1250 ppm). An LEL of 100 ppm (2.5 mg/kg bwt) was established for reduced red blood cell parameters including Hct, Hgb, and RBC. A NOEL was determined to be 0.76 mg/kg day (20 ppm).

A rat teratology study using gavage dosing did not provide evidence of a teratologic effect. The LEL is 300 mg/kg and is based on the fetotoxic end point of delayed ossification. The

NOEL is 30 mg/kg. Maternal toxicity was noted at the 300 and 600 mg/kg dosage levels.

A teratology study in the rabbit using Simazine at doses as high as 200 mg/kg administered by gavage did not provide evidence of teratogenicity in the test animals. A significant increase in skeletal variations (not considered terata)was noted only at the maternally toxic dose of 200 mg/kg (HDT). The NOEL for fetotoxic effects including reduced fetal weights was 75 mg/kg bwt, a maternally toxic dose.

A 3-generation reproduction study in rats using Simazine in the diet did not provide evidence of significant reproductive effects from Simazine exposure.

A replacement study is in progress.

A Salmonella (Ames Assay) was considered acceptable and negative by the Agency. Though several other mutagenicity studies have been submitted, technical difficulties have prevented their acceptance as adequate. Additional studies are required by the Agency.

Rat metabolism studies using ^{14}C-labeled Simazine show that Simazine is preferentially excreted by the urinary route when low doses are used.

^{14}C-Residues (2-12%) may remain in test animals. Since Simazine binds to hemoglobin in rodents, studies in other mammalian species are required by the Agency.

Dermal absorption studies indicate that over a 24-hour period of time less than 1 percent of an applied dose was

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actually absorbed. However, from 10 to 20 percent of the lowest dose was potentially absorbable since it was not dislodged by washing with soap and water.

Plant metabolites have not been completely enumerated in some of the older pesticide submissions. If the complete metabolite profiles indicate that some of these metabolites are not formed in mammalian species, further toxicological studies may be required.

B. Toxicology Profile

81-Series - Acute Toxicity and Irritation Studies

81-1 - Acute Oral

Data exist to indicate that an acute oral LD_{50} for technical Simazine in rats exceeds 5000 mg/kg (MRID No.00148897). The Toxicity Category for the chemical based on these data is IV. Although No further studies are required, additional data concerning the purity of the test material are required for evaluation.

This requirement remains a data gap.

81-2 - Acute Dermal

Data are available on the acute dermal LD_{50} of technical grade Simazine (MRID No. 00148898). Rabbits were not killed when exposed to single doses of 2 g/kg. This value places the test material into Toxicity Category III. Although additional studies are not required, this remains a data gap until further identification with regard to purity is submitted and evaluated.

81-3 - Acute Inhalation

Several studies have been submitted which indicate difficulty in attaining high concentrations of technical Simazine test material in inhalation test chambers. The highest dose tested

(MRID 00148899) was adequate to show that the 4-hour exposure LC_{50} was greater than 1.71 mg/L by actual concentrations. Further data on the purity of the test material are required but the studies enable technical Simazine to be labeled as a Toxicity Category III chemical.

Although further studies are not required this remains a data gap until further information on the purity of the active ingredient is provided to the Agency.

81-4 - Primary Eye Irritation

Data are available to indicate that technical Simazine is only a slight irritant to the eyes with a Toxicity Category of IV (MRID No. 00148900).

Further studies are not required to evaluate eye irritation, but this remains a data gap until the purity of the active ingredient (ai) used in the study is submitted and reviewed.

81-5 - Primary Dermal Irritation

Data are available which show that technical grade Simazine has low dermal irritative potential (MRID No. 00148901). Rabbits were exposed for 4 hours and exhibited only a primary irritation score (PIS) of 0.2 indicating very slight irritation to the skin and that technical grade Simazine is in Toxicity Category IV for dermal irritation effects.

Further studies are not required to evaluate dermal irritation but this remains a data gap until further data concerning the

purity of the test material is submitted and evaluated.

81-6 - Dermal Sensitization

Data have been submitted to the Agency that suggest that technical Simazine probably has a very low sensitizing potential (MRID No. C0142902). However, further information has been requested to more fully evaluate the study.

This is a data gap.

81-7 - Acute Delayed Neurotoxicity

No data have been submitted concerning the acute neurotoxic effects of Simazine. This test is required only for compounds or metabolites that are cholinesterase inhibitors. Simazine is not such a chemical; therefore, a study is not required.

82 Series - Subchronic Testing

82-1 - 90-Day Feeding - Rodent

A 13-week (90-day) feeding study in Sprague-Dawley rats [illegible] dosage of [illegible], 200, 2100 or 4000 parts per million (ppm) (approximately [illegible], 10, 100, or 200 mg/kg bwt) of Simazine in the diet (MRID No. 00143269). The study used 10 animals per sex in [illegible] test and control group with exposure to diet and water on an ad libitum basis. Examinations included body weight, food intake, blood chemistry, urinalysis, and hematology determinations. Organ weights were determined and gross and histological evaluations at study termination were made.

Reduced food intake and [illegible] body weight gains were seen

at dosages of 200 ppm and above. A no-observed-effect level (NOEL) was not established at the lowest dose tested, 200 ppm. The lowest-effect level (LEL) in the study is 200 ppm (the lowest dose tested) based on reduction in erythrocyte counts and elevated cholesterol.

Although a NOEL was not established for the study and the study is core- supplementary, further testing in a subchronic rodent study is not required because there is a core-minimum 2-year rat feeding study that supercedes these results.

82-1 - 90-Day Feeding - Nonrodent

An acceptable subchronic study (MRID No. 00146655) using four dogs of each sex in four groups exposed the test animals to 0, 200, 2000 or 4000 ppm (approximately 0, 5, 50, or 100 mg/kg bwt) of Simazine in the diet and water on an ad libitum basis for 91 days. Observations for appearance, mortality, and signs of toxicity were reported daily. Hematological and clinical chemistry determinations were recorded after 44 and 92 days of exposure. Histological evaluation of a full range of tissues was made on the animals.

Body weight losses were more severe in the females than in males at the mid and high doses. Feed consumption was reduced in both sexes at the mid and high doses by greater than 20 percent from controls. A NOEL was 200 ppm (5 mg/kg/bwt/day) in the diet. The LEL of 2000 ppm was based on reduced serum albumin levels and increased serum globulin values as well as decreased body weights

No further subchronic nonrodent studies are required.

82-2 - Subchronic Dermal (21-Day)

There are sufficient data to evaluate toxicity from a core-guideline 21 day dermal exposure to albino rabbits (MRID No. 00005767). Four test groups each containing 10 rabbits/sex were dermally exposed to 0, 10, 100 or 1000 mg/kg technical grade Simazine for 6 hours for 5 days a week for 3 weeks. Preshaved skin on the back was exposed to test material slightly moistened with saline and imperviously wrapped. The exposures produced no systemic toxicity as evidenced by normal serum chemistry or hematological determinations.

Ulcerative dermatitis was rarely observed (3/80). The NOEL was established at greater than 1000 mg/kg/bwt (HDT).

Additional subchronic dermal exposure studies are not required.

82-3 - Subchronic Dermal (90-Day)

No data are available on the 90-day subchronic dermal toxicity of Simazine. A study is not required for the present use pattern.

82-4 - Subchronic Inhalation (90-Day)

No data are available on the 90-day subchronic inhalation toxicity of Simazine. A study is not required for the present use pattern.

82-5 - Subchronic Neurotoxicity

No data are available on the 90-day subchronic neurotoxicity of Simazine. Since an acute neurotoxicity study is not required and simazine is not a cholinesterase inhibitor, further delayed neurotoxicity studies on Simazine are not required.

83 Series - Chronic and Long-Term Studies

83-1 - Chronic Toxicity - Podent

Adequate data are available in a 2-year chronic feeding study in the Sprague-Dawley strain of rat, exposed to test doses of 0, 10, 100, or 1000 ppm of Simazine in the diet calculated to be 0, 0.41, 4.17, 45.77 and 0, 0.52, 5.34, 63.1 mg/kg bwt in males and females respectively (MRID No. 40614405). Each dose group contained 50 animals per sex and an additional 30 animals/ sex/group for various clinical examinations. There was significant body weight reductions at the highest doses. Hematological indices such as Hct, Hgb, and RBC were reduced in females at 100 ppm (5.3 mg/kg) and higher. Reduced blood glucose levels were noted in females at 5.34 and 63.1 mg/kg.

The data are sufficient to establish a NOEL of 0.52 mg/kg and an LEL of 5.3 mg/kg for the study based on body weight gain depression, decreased serum glucose levels and hematological changes (reduced Hct, Hgb, RBC) in female rats. The study is classified as Minimum for chronic toxicity.

A repeat study is not required.

83-1 - Chronic Toxicity - Nonrodent

A 1-year dog study completed in 1988 is considered adequate to determine chronic toxicity due to technical Simazine (MRID No. 40614402).

Four animals/sex/dose were fed diets containing 0, 20, 100, and 1250 ppm for 1 year which results in approximate doses of 0, 0.68, 3.4, and 43 mg/kg/day for males and similar levels for females, respectively. Hematology, parameters and body weight changes were noted with an LEL = 100 ppm (3.6 mg/kg) established in females. A NOEL was determined as 20 ppm or 0.76 mg/kg/day.

Toxicity was reported in males at the highest dose tested as decreased body weight gain, and hematological changes.

No further chronic toxicity studies are needed for registration requirements.

83-2 - Oncogenicity Study - Mouse - Second Species

Data are considered adequate in a (1988) 95-week oral toxicity/oncogenicity study in mice (MRID No. 40614404). The study used CD-1 mice fed technical grade Simazine in the diet at 0, 40, 1000, or 4000 ppm which equates to 0, 5.3, 131.5, and 542 mg/kg day for males and 0, 6.2, 160, and 652.1 mg/kg/day for females. The study showed toxicity to both sexes at 1000 ppm and above characterized by the loss of body weight gain and reduced red blood cell counts. Neoplastic changes from exposure to the chemical were absent. The LEL was 1000 ppm based on decreased weight gain. The NOEL was 40 ppm, approximately 6 mg kg/ day.

An additional oncogenicity study in this specie is not required.

83-2 - Oncogenicity - Rat

Data are adequate from a recent (1988) oncogenicity study (see 83-1) using Sprague-Dawley rats to indicate that exposure for 2 years induces mammary tumors in female rats (MRID No. 40614405). Groups of 50 animals per sex were placed on study for the oncogenicity testing segment. An additional 30/sex/group were placed on study to evaluate the toxicity portion of the study. Histopathology was examined on all available animals on study. Test dose levels included 0, 10, 100, and 1000 ppm equating to 0, 0.5, 5.3, and 61.1 mg/kg bwt/day for females and 0, 0.41, 4.17, and 45.9 mg/kg bwt/day in males of technical Simazine in the diet. Significant reductions in body weight gain and reduced hematologic parameters were observed in the females at 100 ppm (5.3 mg/kg/day) and above.

Statistical analysis of tumors reported in the study found that there were significant increases in mammary gland carcinomas at the mid and high doses. Additionally, an increase in fibroademonas was also found at the highest dose level. Pituitary tumors were also found to be increased in the mid and high dose female rats.

Other tumors of importance appeared to be the slight but non statistically significant increase in male rat liver tumors at 1000 ppm of Simazine in the diet.

An additional oncogenicity study in [redacted] species is not required.

Further evaluation of the oncogenicity of Simazine has been made by the HED Peer Review Panel. See Section E for a discussion of the results of that review and conclusions.

83-3 - Teratogenicity - Rat

A teratology study in the Sprague-Dawley COBS rat (MRID No. 40614403) was presented to the Agency in fulfillment of the registration requirement.

The rat study (ca. 1986) used 25 young adult females in each of four study groups exposed to gavage doses of 0, 30, 300, or 600 mg/kg/day on days 6 through 15 of the gestation period. Pregnancy was accomplished by natural breeding. The dams were observed for signs of toxicity including weight losses and food consumption.

There were significant weight gain decreases in the dams at the 300 and 600 mg/kg levels. Food consumption decreased at doses as low as 30 mg/kg. Fetotoxicity at 300 and 600 mg/kg was demonstrated by delayed skeletal ossification in a wide range of osseous sites including ribs, teeth, head, and vertebrae. A NOEL for the above fetal effects was 30 mg/kg with the LEL established at 300 mg/kg.

A maternal toxicity NOEL was 30 mg/kg and the LEL was 300 mg/kg based on decreased food consumption.

There were no indications of a teratogenic effect.

This study is incomplete and additional information is

required before fully supporting registration requirements.

83-3 - Teratogenicity - Rabbits

A teratogenicity study (MRID No. 00161407) in rabbits gavaged with technical Simazine is adequate to show that Simazine possesses low teratogenic potential. Rabbits were treated with 0, 5, 75, and 200 mg/kg/bwt Simazine on days 7 through 19 of gestation. A significant reduction in mean fetal weights was seen indicating fetotoxicity at 200 mg/kg. Skeletal variations were also increased at this dose. A NOEL for fetal effects was 75 mg/kg with an LEL of 200 mg/kg. The maternal NOEL was 5 mg/kg and the LEL was 75 mg/kg based on tremors, abortions, and decreased body weight gains.

The study is adequate for registration requirements.

83-4 - Reproduction

An inadequate three-generation reproduction study (MRID No.00023365, 00080631) in rats suggests that Simazine does not cause adverse changes in generations exposed to Simazine. The study (ca. 1965) of Simazine in white rats used 20 males and 20 females as F_0 parents fed 0 or 100 ppm (approximately 5 mg/kg) of Simazine in the diet as 80W simazine for 26 weeks. All further litters and generations were studied in 20 females and 10 males for breeding and an additional test group at 50 ppm was added.

The F_{3b} rats were autopsied with weights of the heart, liver, and kidneys determined in two males and females from each litter.

Tissues from only one of each sex/litter were examined histologically.

Growth rates were similar in all three generations of Simazine treated and control rats except that F_1b males exhibited a lesser weight gain than controls.

Reproductive performance was similar with regard to live birth weights, number of live weanlings per litter, and mean weanling weights. The number of litters per group at both 50 and 100 ppm were similar to controls.

The individual data on pup weights and low numbers of histologically evaluated tissues and number of individual findings missing from the report leads the reviewer to conclude that the study by recent standards does not support the registration requirements. This is considered a data gap by the Agency however, the Agency is aware that a replacement study is nearing completion and is to be submitted by 1990.

84 Series - Mutagenicity Testing

84-2 - Gene Mutation

A Salmonella/mammalian microsome mutagenicity assay (Ames assay) tested technical Simazine in 5 doses ranging from 10 to 250 ug per plate. Due to precipitation limits, the maximum dose tested was acceptable. No level was cytotoxic or mutagenic in the assay (MRID No. 40614406).

No further gene mutation studies are required for technical Simazine.

84-2 - Chromosomal Aberration

A study using a human lymphocyte cytogenetic assay (MRID No. 40614407) at doses of 6.25, 12.5, 25, 50, or 100 ug/mL of technical Simazine did not adequately evaluate the aberrational potential of Simazine.

A replacement study is required for fulfillment of registration requirements.

84-2 - Other Mechanisms of Mutagenicity - Unscheduled DNA Repair

Primary rat hepatocyte cultures exposed to 0.4, 2, 10, and 50 ug/mL of technical Simazine did not provide adequate information to evaluate the mutagenic potential of the test chemical (MRID No. 40614408). Insufficient information was supplied with the study report.

A replacement study is required for fulfillment of registration requirements.

Special Testing

85-1 - General Metabolism

There is an acceptable study (MRID No. 00143266) that indicates that Simazine is excreted principally via the urine at low doses (0.5 mg/kg). By using ^{14}C labeled Simazine it was found that 94 to 99 percent of elimination occurred in 48 to 72 hours with a half-life (t1/2) of 9 to 15 hours. Heart, lung, spleen, liver, and kidney appear to be principal sites of retention of the

residual radioactivity. It was found that cysteine residues in hemoglobin exhibited a high affinity for the triazine ring. Although it has been suggested that the phenomenon is apparently unique to rodents, HED does not have data to support this hypothesis.

No additional data are required for the topic of general metabolism.

85-3 - Dermal Absorption - Rat

Sufficient data are available in MRID No. 40614409 of a ^{14}C-Simazine study in rats to indicate that ^{14}C-Simazine actually enters the rat body to less than 1 percent of the applied dose within 24 hours. However, when exposures of 1 and 5 mg/rat were examined, it was found that up to 30 to 40 percent of the dose remained in the skin as potentially absorbable.

85-X -Special Study

Additional data are required in other mammalian species to support the hypothesis that red blood cell binding is uniquely limited to rodents.

C. Data Gaps

Simazine is registered for many types of uses including terrestrial food and nonfood, aquatic food and nonfood, and forestry. Therefore, the following Guidelines toxicology studies can be required for registration.

81 Series - Acute Toxicity and Irritation Studies

81-1 - Oral LD_{50} - Rat

81-2 - Dermal LD_{50}

81-3 - Inhalation LC_{50} - Rat

81-4 - Primary Eye Irritation

81-5 - Primary Dermal Irritation

81-6 - Dermal Sensitization

82 Series - Subchronic Testing

82-1 - Subchronic Oral (Rodent, Nonrodent)

82-2 - Subchronic Dermal (21-day)

83 Series - Chronic and Long-Term Studies

83-1 - Chronic Feeding (rodent, nonrodent)

83-2 - Oncogenicity (2 species)

83-3 - Teratogenicity (2 species)

83-4 - Reproduction (2-generation)

84 Series - Mutagenicity

84-2 - Mutagenicity Tests

- Gene mutation
- Chromosomal aberration
- Direct DNA damage
- Other tests

85 Series - Special Studies

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85-1 - Metabolism

85-3 - Dermal Absorption

85-X - Special (not specified)

Based on this assessment of the toxicology data base for Simazine technical, the following toxicology studies have been identified as presently existing data gaps and are required.

Acute Testing

81-1 - Oral LD_{50} - Rat

81-2 - Dermal LD_{50}

81-3 - Inhalation LC_{50} - Rat

81-4 - Primary Eye Irritation - Rabbit

81-5 - Primary Dermal Irritation

81-6 - Dermal Sensitization

83 Series - Chronic Testing

83-3 - Teratogenicity - Rat

83-4 - Reproduction - 2-Generation

84 Series - Mutagenicity Testing

84-2 - Chromosomal aberrations

84-2 - Other mechanisms of mutagenicity (unscheduled DNA synthesis)

85 Series - Special Testing

85-X - Mammalian species red blood binding studies with Simazine (in vitro and in vivo tiered).

D. Tolerance Assessment (Rfd)

Simazine has been registered for both food and feed uses as well as nonfood uses with established tolerances under 40 CFR 180.213 and 180.213(a). Therefore, an ADI (Rfd) is required to be established for this chemical. The tolerances are based upon the residues of the parent compound and its triazine metabolites.

An ADI(acceptable daily intake) was established in the initial registration standard and was based upon the NOEL of 5 mg/kg/day in a 3-generation reproduction study in the rat. Since that initial standard, several studies have been reviewed and considered in establishing an Rfd (new nomenclature for an ADI). The 2-year rat chronic feeding study provides data to establish a NOEL of 0.5 mg/kg/day for body weight gain and hematological changes in females.

A 1-year subchronic/chronic dog study provides data to establish a NOEL of 0.76 mg/kg/day. A three hundred-fold uncertainty factor was applied to the lowest NOEL of 0.5 mg/kg/day thus establishing a PADI (RFD) of 0.002 mg/kg/day.

The additional 3 fold factor is used to provide safety from the lack of an adequately performed reproduction study. The PADI (RFD) has been verified by the Agency RFD Work Group (June 15, 198[illegible]).

When the reproduction study now in progress has been submitted and reviewed, the additional 3 fold factor may be deleted.

[illegible]. Toxicological Issues

Several toxicological issues exist for the registration of Simazine including those of oncogenicty, binding of Simazine to the hemoglobin molecule of mammals, and the lack of toxicological testing of metabolites found in plants but not formed in mammalian metabolism of Simazine.

Oncogenicity - Weight of the evidence

Oncogenicity was [illegible] in the rat study. Female rats [illegible] to be slightly more sensitive to exposure of Simazine in the diet than the males. A previous [illegible] inadequate chronic rat [illegible] showed increased incidences of mammary tumors. The new acceptable chronic rat study also [illegible] was an increased incidence of mammary tumors. The problem was presented to the HED Peer Review Group [illegible] on [illegible], 1989 (see Peer Review document in [illegible]). The HED Peer Review Committee concluded that the highest dose was exceeding an MTD for the female rat [illegible] and that the chemical should be classified [illegible]. [illegible], the [illegible] risk, [illegible] was [illegible] [illegible] in human equivalents. The [illegible] based [illegible] the incidence of mammary gland carcinomas in [illegible].

[illegible]

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The mammary tumor response was consistent with that seen in other studies with triazines. Evidence for a mode of action for oncogenicit was not available. However, the Committee concluded that there was some (open literature-nonreviewed) evidence of genotoxicity. (Submission to the Peer Review Committee is attached) The final Peer Review Document is in preparation and unavailable for inclusion at this printing, therefore a draft document is provided as an attach-

2. Hemoglobin Binding

A second toxicological issue is the increased affinity of hemaglobin binding by the triazine ring of Simazine in mammals. Both the 1-year dog and 2-year chronic rat study suggest that compound-related effects occur in hematologic parameters.

Additional binding studies will help delineate possible hazard of blood elements exposed to the triazines.

3. Metabolites

Additional studies on the metabolites of Simazine found in foods which are not formed by mammals may be necessary when the Dietary Exposure Branch of Health Effects Division review the residue data they have requested.

F. Toxicology Summary Tables

Table A
Generic Data Requirements for Simazine

Data Requirement	Composition[1/]	Use Patterns[2/]	Does EPA Have Data To Satisfy This Requirement? (Yes, No or Partially)	Bibliographic Citation (MRID No.)	Must Additional Data Be Submitted Under FIFRA Section 3(c)(2)(B)?[3/]
§158.135 Toxicology					
ACUTE TESTING					
81-1 - Acute Oral - Rat	TGAI	ABCDG	NO	00148897	Yes[4/]
81-2 - Acute Dermal	TGAI	ABG	NO	00148898	Yes[4/]
81-3 - Acute Inhalation - Rat	TGAI	ABG	NO	00148899	Yes[4/]
81-4 - Eye Irritation - Rabbit	TGAI	ABG	NO	00148900	Yes[4/]
81-5 - Dermal Irritation - Rabbit	TGAI	ABG	NO	00148901	Yes[4/]
81-6 - Dermal Sensitization - Guinea Pig	TGAI	ABG	NO	00148902	Yes[4/]
81-7 - Acute Delayed Neurotoxicity - Hen	TGAI	ABG	NO	--	No[5/]
SUBCHRONIC TESTING					
82-1 - 90-Day Feeding - Rodent	TGAI	ABCDG	No	00143265	No[6/]
Nonrodent	TGAI	ABCDG	Yes	00146655	No
[illegible]-Day Dermal	TGAI	ABG	Yes	00005767	No
[illegible]-Day Dermal	TGAI	ABG	No	--	No[6A]
82-4 - 90 Day Inhalation	TGAI	ABG	No	--	No[6A]
82-5 - 90-Day Neurotoxicity	TGAI	ABG	No	--	No[5,6A]

Table A
Generic Data Requirements for Simazine

Data Requirement	Composition[1/]	Use Patterns[2/]	Does EPA Have Data To Satisfy This Requirement? (Yes, No or Partially)	Bibliographic Citation (MRID No.)	Must Additional Data Be Submitted Under FIFRA Section 3(c)(2)(B)?[3/]
§158.135 Toxicology (cont'd)					
CHRONIC TESTING:					
83-1 - Chronic Toxicity					
- Rodent	TGAI	ABCDG	Yes	40614405	No
- Nonrodent	TGAI	ABCDG	Yes	40614402	No
83-2 - Oncogenicity Study					
- Rat	TGAI	ABCDG	Yes	40614405	No
- Mouse	TGAI	ABCDG	Yes	40614404	No
83-3 - Teratogenicity					
- Rat	TGAI	ABCDG	NO	40614403	Yes[4/]
- Rabbit	TGAI	ABCDG	Yes	00161407	No
83-4 - Reproduction	TGAI	ABCDG	No	00023365 00080631	Yes[7/]
MUTAGENICITY TESTING					
84-2 - Gene Mutation	TGAI	ABCDG	Yes	40614406	No
84-2 - Chromosomal Aberration	TGAI	ABCDG	No	40614407	Yes[3/]
84-2 - Other Mechanisms of Mutagenicity	TGAI	ABCDG	No	40614408	Yes[3/]
SPECIAL TESTING:					
85-1 - General Metabolism	PAI or PAIRA	ABCDG	Yes	00143266	No
85-2 - Domestic Animal Safety	Choice	N/A	--	--	--

Table A
Generic Data Requirements for Simazine

Data Requirement	Composition[1/]	Use Patterns[2/]	Does EPA Have Data To Satisfy This Requirement? (Yes, No or Partially)	Bibliographic Citation (MRID No.)	Must Additional Data Be Submitted Under FIFRA Section 3(c)(2)(B)?[3/]
§158.135 Toxicology					
SPECIAL TESTING (cont'd)					
85-1 - Dermal Absorption	TAI or PAIRA	ABCDG	Yes	40614409	No
85-X - Mammalian RBC Binding Studies	PAIRA	ABCDG	No	--	Yes[7/]

1/ Composition: TGAI = Technical Grade Active Ingredient; PAI = Pure Active Ingredient; PAIRA = Pure Active Ingredient Radiolabeled; Choice = Choice of several test substances determined on a case-by-case basis.
2/ The use patterns are coded as follows: A = Terrestrial, Food Crop; B = Terrestrial, Nonfood; C = Aquatic, Food Crop; D = Aquatic, Nonfood; E = Greenhouse, Food Crop; F = Greenhouse, Nonfood; G = Forestry; H = Domestic Outdoor; I = Indoor; IP = Industrial Preservative.
3/ Unless otherwise specified, data must be submitted no later than 6 months after publication of this Standard.
4/ The studies are lacking some data which upon receipt and review may elevate the status of the study to fully acceptable as registration requirement.
5/ This test is required only for compounds which are organophosphate inhibitors of cholinesterase or related to such inhibitors or metabolites of such inhibitors. Simazine is not an organophosphate, therefore a study is not required.
6/ This study is not required under the existing use pattern since longer term study supercedes these results.
6A/ This study is not required under the existing use pattern.
7/ Unless otherwise specified, data must be submitted no later than 1 year after publication of this Standard.

G. Bibliography

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00148900 Rosenfeld, G. (1985) Primary Eye Irritation Study in Rabbits: Test Article: Simanex Tech (Simazine): Study #1221D. Unpublished study prepared by Cosmopolitan Safety Evaluation, Inc. 17 p.

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H. One-Liners

CITATION	MATERIAL	ACCESSION/ MRID NO.	RESULTS	TOX CAT	COREGRADE/ DOCUMENT#
Acute Inhalation LC50 Species: rat Cosmopolitan Safety Eval. 1221C; 3/25/85	Simazine Tech (purity not stated)	00148899	LC50 > 1.71 mg/L (4 hr.) Dose was the max. maintained in breathing zone. may be upgraded with purity data submission. Observations were for 14 days. Signs of toxicity were: body wt. loses, decr. of activity, wetting of muzzle during exposure. No deaths occurred. MMAD was 1.1 microns. Nominal conc. was 14.7 mg/L.	3	Supplementary 007240
Primary eye irritation Species: rabbit Cosmopolitan Safety Eval. 1221D; 3/25/85	Simazine Tech (purity not stated)	00148900	Very slightly irritating to the eyes. redness was only at 1 hr. in all 6 animals and resolved by 24 hrs.	4	Supplementary 007240
Primary dermal irritation Species: rabbit Cosmopolitan Safety Eval. 1221E; 3/25/85	Simazine Tech (purity not stated)	00148901	PIS = 0.2; very slightly irritating to the skin of unsexed animals. Exposure was 4 hrs. under impervious wrap. Erythema was never > 1 grade. Complete resolution by 72 hrs. All effected by 45 min. May be upgraded with purity data.	4	Supplementary 007240
Dermal sensitization Species: guinea pig Cosmopolitan Safety Eval. 1221F; 3/25/85	Simazine Tech. (purity not stated)	00148902	10 male pigs were tested. 0.5 g in paraffin oil was applied 6 hr/day per 3 weeks under occlusive bandage. Results: 2 exposed to Simazine produced inconsistent results in the induction - no irritation was noted at challenge. Question: data are absent to show whether paraffin oil allows Simazine to enter the animal.	+	Supplementary 007240
Acute oral LD50 Species: rat Cosmopolitan Safety Eval. 1221A; 3/25/85	Simazine Tech. 1181 (purity not stated)	00148897	LD50 > 5.0 g/kg (ODT). Results: 1/5 M died on day 6; 2/5 F died on day 6. May be upgraded with submission of purity.	4	Supplementary 007240
Acute Dermal LD50 Species: rabbit Cosmopolitan Safety Eval. 1221B; 3/25/85	Simazine Tech. (purity not stated)	00148898	LD50 > 2.0 g/kg "limit test"; 5/6 young rabbits were exposed using an occluded dressing for 24 hrs. No signs of toxicity other than erythema and edema which resolved by day 7.	3	Supplementary 007240

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CITATION	MATERIAL	ACCESSION/ MRID NO.	RESULTS	TOX CAT	CORE GRADE/ DOCUMENT#
Oncogenic risk assessment Species: rat Ciba-Geigy Ltd. 2-011-09; 4/12/88	Simazine tech	406144-05	Two Year Rat Chronic/Onco Study: Qualitative Risk Assessment Male rats: significant decrease mortality trend and 1000 ppm had a significant less mortality. No trends for liver tumors. Liver carcinoma was significantly increased at 100 ppm. Combined liver adenoma/carcinoma significant increased at 1000 ppm. Thyroid C-cell tumors no trend or pair-wise difference. Kidney tubule carcinomas and combined adenoma/carcinoma had a significant trend but no pair-wise difference.		006948
Oncogenic risk assessment Species: rat Ciba-Geigy Ltd. 2-011-09; 4/12/88	Simazine tech	406144-05	Two Year Rat Chronic/Onco Study: Qualitative Risk Assessment Female rats: significant trend in mortality at 100 ppm and 1000 ppm dose groups; significant increase over controls. Mammary carcinomas had a significant trend and 100 ppm and 1000 ppm significantly increased over controls. Combined mammary adenomas/carcinomas trend was significant and there was a significant increase over controls at 1000 ppm. Fatal pituitary gland adenomas, carcinomas alone and combined had a significant trend. Adenomas and combined were significantly increase over controls for 100 and 1000 ppm. Carcinomas at 100 ppm was significantly increase over controls. Kidney tubule adenomas had a significant dose trend.		006948 007240
Oncogenic risk assessment Species: rat Dynamac# 1-16 10/18/88	Simazine		Unit Risk, Q1* = 1.20 X 10exp-1 (mg/kg/day)exp-1 in human equivalents, based on mammary gland carcinomas in female Sprague-Dawley rats with dose levels of 0, 10, 100, & 1000 ppm. Analyzed by Weibull83 because females had significant increase mortality with dose increments of simazine.		007309
Metabolism Species: rat Stanford Research Inst. ABR 86032; 4/30/86	Simazine	262646 00143266	At the low dose of administration (0.5 mg/kg) of 14C-radiolabeled simazine, the principal route of excretion was via the urine; however, at the higher dose (200 mg/kg) the principal route of excretion was via the feces. Significant radioactive residues remained in the tissues of the rat for extended periods of time. Results indicate that 94 to 99 percent of the elimination of radioactive material occurred within 48 to 72 hrs. with a half-life of 9 to 15 hrs. Elimination of the remaining radioactivity exhibited 21-32 hour half-life values. Heart, lung, spleen, kidney, and liver appear to be principal sites of retention of radioactivity. However, erythrocytes concentrated radioactivity to higher levels than did other tissues, perhaps due to high affinity of the triazine ring for cysteine residues of hemoglobin, a phenomenon apparently unique to rodent species.		005641 007240

00240

CITATION	MATERIAL	ACCESSION/ MRID NO.	RESULTS	TOX CAT	CO-GRADE/ DOCUMENT#
Feeding-13 week Species: rat Ciba Geigy Pharmaceutical, Eng 85018; 4/10/85	Simazine tech; batch FL 840988, 97.5% pure	257693 00143265	NOEL < 200 ppm (LDT); (reduction in erythrocyte (m & f) and leucocyte (m) counts; elevated cholesterol and inorganic phosphate levels (m & f); renal calculi in 3/20 rats (m & f). MTD < 2000 ppm; seriously affected nutrition of treated rats (m & f). Dose levels: 0, 200, 2000 and 4000 ppm in Sprague-Dawley (CRL:COB CD (SD) BR)		Supplementary 004656 007240
Feeding-13 week Species: dog Ciba Geigy Pharmaceutical, Eng 85022; 4/12/85	Simazine tech; batch FL 840988; 97.5% pure	257692 00146655	NOEL = 200 ppm. LEL = 2000 ppm; (reduced albumin levels and increased globulin levels (m), and elevated urinary specific gravity (m) and Ketone levels). MTD < 2000 ppm; seriously affected nutrition of treated dogs (m & f). Dose levels: 0, 200, 2000 and 4000 ppm in beagles		Minimum 004656 007240
Registration standard	Simazine		Tox Chapter · 1983 Tox Chapter to SRR · 1989		004255 007240
Dermal absorption Species: rat Wil Research Lab ABR-88042; 3/30/88	Simazine-C14 label, 96-98% radio purity	406144-09	Doses: 0.1 and 0.5 mg/cm2 exposures were for: 2, 4, 10 and 24 hrs. Actual dermal absorption was less than 1% after 24 hrs. Between 11% and 20% of low dose and 31% and 41% of high dose remained in the skin after soap and water wash as potentially absorbable.		Acceptable 007240
Mut. Chrom. aberr. in vitro Species: human lymphocytes Ciba Geigy Ltd.,Switz. 87107; 3/24/88	Simazine Tech. 99.6% pure	406144-07	Human lymphocytes were tested at 6.25, 12.5, 25, 50 and 100 ug/ml with/without S9 activation. Harvest was at 43.5 hr. but should have been at 24 hr.		Unacceptable 007240
Mut. gene. Ames Species: salmonella Ciba Geigy, N.J. 87038; 7/8/87	Simazine Tech. (a product) purity not stated.	406144-06	Organisms tested were: S. typh.: TA 1535, TA 100, TA 1538, TA98, TA 1537 with and without mammalian microsomal S9 activation in DMSO. Tested at 5 doses from 10-250 ug/plate. NOT precipitated. NOT was nontoxic & not mutagenic in study. Positive controls: B-a-pyrene, 9-aminoacridine, Na azide, B-naphthylamine, 3-methyl-cholanthrene.		Acceptable 007240
Mutagenic-unscheduled DNA synt Species: rat prim. hepatocyte Ciba-Geigy Ltd.,Switz. 830640; 12/20/83	Simazine Tech. 99.6% in DMSO	40614408	Rat liver cells culture were tested at: 0.4, 2, 10, 50 ug/ml for 5 hrs, did not show increased radio granules. Pos. control was DMN. The exposure was too short (should have been 18 hrs.)		Unacceptable 007240

007210

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CITATION	MATERIAL	ACCESSION/ MRID NO.	RESULTS	TOX CAT	COREGRADE/ DOCUMENT#
Teratology Species: rabbit Ciba-Geigy Ltd. 82-83; 5/17/85	Simazine Tech 97%; Lot 821846	252938 260651 00161407	Levels tested by gavage in New Zealand White: 0, 5, 75 & 200 mg/kg Teratogenic NOEL > 200 mg/kg (HDT). Maternal NOEL = 5 mg/kg Maternal LEL = 75 mg/kg (tremors, abortions & decreased body weight gain & food consumption). Feto toxic NOEL = 75 mg/kg Fetotoxic LEL = 200 mg/kg (reduced mean fetal weight and increased skeletal variations.) A/D ratio = 5/75 = 0.06		Supplementary 004535 Guideline 005127 007240
Reproduction 3 generation Species: rat Woodard Research 1965	Simazine 80W	00023365 00080631	Reproductive NOEL > 100 ppm (only dose tested). Reevaluation for 1989 Reg. Std. The study is downgraded. Not able to be upgraded. Too few animals were examined histologically and possibly sterile males were not evaluated. A NOEL can not be determined due to incomplete sampling and evaluation of males, but may be > 100 ppm (HDT).		Minimum 003689 Supplementary 007240
Teratology Species: rat Ciba Geigy Ltd 85/110(MIN 8[illegible], 4/7/86	Simazine Tech. (purity not stated but was determined as 97.5% from other studies)	40614403	CD8S rats were gavaged with: 0, 30, 300 and 600 mg/kg in 2% CMC. 25 F per group on gest. days 6-15. Maternal tox LEL = 300 mg/kg for decr. body wt. & wt. gain. Maternal NOEL = 30 mg/kg. Fetal effects LEL = 300 mg/kg for increased number of centra/vertebrae & incomplete ossification noted in the head, teeth, vertebrae & sternebrae. Fetotoxic NOEL = 30 mg/kg. Additional information is required and study may be upgraded. A/D ratio = 1.0.		Supplementary 007240
Feeding/oncogenic - 12 months Species: mice Ciba Geigy Ltd. 84-2121; 4/4/88	Simazine Tech (purity not reported) batch #LB40-988	40614404	Crl:CD1(ICR) BR mice fed diets of 0, 40, 1000 & 4000 ppm (5.2, 131.5, 542 mg/kg/day (M); 6.2, 160.0, 652.1 mg/kg/day (F)). NOEL = 40 ppm (5.7 mg/kg M), based on reduced body wt. gain & reduced red blood cell counts in both sexes. LEL = 1000 ppm (131.5 mg.kg M). Oncogenicity was absent at up to 4000 ppm (542 mg/kg M)		Guideline 007240
Feeding/oncogenic 2 year Species: rat Ciba-Geigy Ltd. 2 011-09; 4/12/88	Simazine Tech 97.5%	40614405	Tested in Sprague-Dawley (Crl:VAF plus CD(SD) Br. strain in diet: 0, 10, 100, 1000 ppm (0.41, 4.17, 45.7 mg/kg/day M; 0.52, 5.34, 63.1 mg/kg/day F). NOEL = 10 ppm. LEL = 100 ppm (for depression of body wt. gain & depression of RBC, HGB, HCT in females. In addition at 1000 ppm body wt. was depressed in males. Oncogenic (F) - mammary tumors at 100 ppm & above. In males - liver tumors at 1000 ppm.		Minimum 007240
Feeding 1 year oral capsule Species: dog Ciba Geigy Ltd. 882001; 3/28/88	Simazine Tech. (usually 97.5%)	40614402	Doses tested in diet to beagles were: 0, 20, 100, 1250 ppm (0.68, 3.41, 42.9 mg/kg/day (M); 0.76, 3.64, 44.9 mg/kg/day (F). NOEL = 20 ppm. LEL = 100 ppm (decr. body wt. gain and decr. RBC, HGB, HCT (females)). In addition at 1250 ppm decr. decr. body wt. gain and decr. (reversible) RBC, HCT in males. Females were more sensitive than males.		Minimum 007240
Dermal-3 week Species: rabbit Bioresearch Inc. 12017; 1/14/80	Simazine Tech 97.6%	00005767	NOEL > 1000 mg/kg (HDT.) Levels tested = 0, 10, 100, 1000 mg/kg		Guideline 001680 007240

00724010

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I. Reference Case

__I.A. REFERENCE DOSE FOR CHRONIC ORAL EXPOSURE (RfDo)

007240

Chemical -- Simazine
CASRN -- 122-34-9
CASWELL -- 740
On-line: 07/11/89

___I.A.1. ORAL RfD SUMMARY

Critical Effect	Experimental Doses*	UF	MF	RfD
Reduction in weight gains; hematological changes in females 2-Year Rat Feeding/ Oncogenicity Study Ciba-Geigy Corp., Pharmaceutical Div., 1988	NOEL: 10 ppm (0.52 mg/kg/day) LEL: 100 ppm (5.3 mg/kg/day)	300	1	2E-3 mg/kg/day

*Conversion Factors: Actual dose tested

___I.A.2. PRINCIPAL AND SUPPORTING STUDIES (ORAL RfD)

McCormick, C.C.; Arthur, A.T.; Green, J.D.
Simazine: 104-Week Oral Chronic Toxicity and Carcinogenicity Study in Rats
Ciba-Geigy Corp., Pharmaceutical Division
Ciba-Geigy Corporation
Study No. 2-011-09; April 12, 1988
MRID No. 40614405

Groups of Sprague-Dawely rats (50/sex/dose level) were given diets ad libitum containing 0, 10, 100 or 1000 ppm (Male: 0, 0.41, 4.2, 45.8 mg/kg/day; Female: 0, 0.52, 5.3, 63.1 mg/kg/day) of technical Simazine for 2 years. All animals were observed daily for clinical symptoms of toxicity and mortality. 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.

Significant reductions in body weight gain and hematologic parameters (RBC, HGB, and HCT) in females at 100 ppm (5.3 mg/kg/day) and in the additional animals, 30/sex/dose, which were included to evaluate toxicity. A significant increase in mammary carcinomas was reported at the mid and high dose (5.3 and 63.1 mg/kg/day) females. Pituitary tumors were also increased in females in the top two dosage groups. A slight but not statistically significant increase in liver tumors in males at the highest dosage (45.8 mg/kg/day) was observed. The NOEL for this study was established at 10 ppm (0.52 mg/kg/day) for the weight changes and hematologic parameters observed in females at 100 ppm (5.3 mg/kg/day)

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___I.A.3. UNCERTAINTY AND MODIFYING FACTORS (ORAL RfD) 007240

UF = 300. An uncertainty factor of 100 was used to account for the inter- and intraspecies differences. An additional UF of 3 was used to account for the lack of an acceptable reproduction study.

MF = 1.

___I.A.4. ADDITIONAL COMMENTS (ORAL RfD)

Data Considered for Establishing the RfD

1) 2-Year Feeding/Oncogenicity - rat: NOEL=10 ppm (0.5 mg/kg/day); LEL=100 ppm (5.3 mg/kg/day) (depression of body weight gains and depression of values for the hematology parameters, RBC, HGB, and HCT in females); In female rats mammary tumors were observed at 100 and 1000 ppm (5.3 and 63.1 mg/kg/day); In male rats formation of liver tumors (hepatocellular adenomas/carcinomas) were observed at 1000 ppm (45.8 mg/kg/day); core grade minimum (Ciba-Geigy Corp., 1988a)

2) 1-Year Feeding - dog: NOEL=20 ppm (0.76 mg/kg/day); LEL=100 ppm (3.6 mg/kg/day) (decreased body weight gain, and decreases in RBC, HGB, HCT, and a nominal increase in platelet count in females); At 1250 ppm (45 mg/kg/day) in females decreases occurred in body weight gain, and in RBC, HGB, and HCT. At 1250 ppm (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 were observed; core grade minimum (Ciba-Geigy Corp., 1988b)

3) 3-Generation Reproduction - rat: Parental NOEL<50 ppm (2.5 mg/kg/day) (LDT; reduced body weight gains in males in the premating periods); core grade supplementary (Reproductive toxicity could not be determined based on lack of histologic evaluations in apparently sterile males in the F1b generation. Up to 33% of the potential paternal stock at 100 ppm did not produce a pregnant female in two successive breeding sessions. The small sample size of the F3b pups examined , and the length of gestation was not determined. Pup and litter weights at 14 and 21 days were not determined. The male and female parents were not examined histologically in any generation) (Ciba-Geigy Corp., 1965)

4) Teratology - rat: Maternal toxicity NOEL=30 mg/kg/day; Maternal toxicity LEL=300 mg/kg/day (decreased maternal body weight and body weight gain, food consumption, and efficiency of food utilization); Developmental toxicity NOEL=30 mg/kg/day; Developmental toxicity LEL=300 mg/kg/day (increased head incompletely ossified, teeth not ossified, centra/vertebrae unossified and/or (additional), rudimentary ribs, presphenoid not ossified, and sternebrae not ossified); core grade supplementary (additional data must be submitted) (Ciba-Geigy Corp., 1986)

5) Teratology - rabbit: Maternal NOEL=5 mg/kg/day; Maternal LEL=75 mg/kg/day (tremors, abortions, and decreased weight gain and food consumption); Fetotoxic NOEL=75 mg/kg/day; Fetotoxic LEL=200 mg/kg/day (reduced mean fetal weight and increased skeletal variations); Teratogenic NOEL>200 mg/kg/day (HDT); core grade guideline (Ciba-Geigy Corp., 1984)

Other Data Reviewed:

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1) Oncogenicity - mice: No evidence of oncogenicity was observed at any dose tested (600 mg/kg/day, HDT); 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. Therefore the nonneoplastic changes were not considered directly related to dosing. The incidence of amyloidosis was high in all groups. The LEL is based on decreased weight gain was 1000 ppm (150 mg/kg/day) and the NOEL 40 ppm (6 mg/kg/day); core grade guideline (Ciba-Geigy Corp., 1988c)

2) 13-Week Feeding - rat: NOEL<200 ppm (10 mg/kg/day) [LDT; reduction in erythrocyte (M&F) and leucocyte (M) counts; elevated cholesterol and inorganic phosphate levels (M&F); renal calculi in 3/20 rats (M&F); core grade supplementary (Ciba-Geigy Corp., 1985a)

3) 13-Week Feeding - dog: NOEL=200 ppm (5 mg/kg/day); LEL=2000 ppm (50 mg/kg/day) [reduced albumin levels and increased globulin levels (M), and elevated urinary specific gravity (M) and ketone levels (M&F)]; core grade minimum (Ciba-Geigy Corp., 1985b)

Data Gap(s): Rat Reproduction Study; Rat Teratology Study

___I.A.5. CONFIDENCE IN THE ORAL RfD

Study: Medium
Data Base: Medium
RfD: Medium

The critical study is of adequate quality and is given a medium confidence rating. Since adequate studies in reproduction and teratology (rat) are lacking, the data base is given a medium confidence in the RfD follows.

___I.A.6. EPA DOCUMENTATION AND REVIEW OF THE ORAL RfD

Registration Standard, September 1985
Registration Files

Agency RfD Work Group Review: 06/24/86, 06/15/89

Verification Date: 06/15/89

___I.A.7. EPA CONTACTS (ORAL RfD)

007240

George Ghali / OPP -- (703)557-7490 / FTS 557-7490

Reto Engler / OPP -- (703)557-7491 / FTS 557-7491

_VI. REFERENCES

Ciba-Geigy Corp., 1988a. MRID No. 40614405
Available from EPA. Write to FOI, EPA, Washington D.C. 20460.

Ciba-Geigy Corp., 1988b. MRID No. 40614402
Available from EPA. Write to FOI, EPA, Washington D.C. 20460.

Ciba-Geigy Corp., 1965. MRID No. 00023365, 00080631
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Ciba-Geigy Corp., 1984. MRID No. 40614404
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Available from EPA. Write to FOI, EPA, Washington D.C. 20460.

J. Draft Peer Review Document

007240

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

DRAFT

OFFICE OF
PESTICIDES AND TOXIC SUBSTANCES

MEMORANDUM

SUBJECT: Peer Review of Simazine

FROM: Esther Rinde, Ph.D. E. Rinde 6/16/89
Science Analysis and
Coordination Branch
Health Effects Division (TS-769c)

TO: James Yowell
Product Manager #23
Registration Division (TS-767c)

The Health Effects Division Peer Review Committee met on May 17, 1989 to discuss and evaluate the weight-of-the-evidence on Simazine with particular reference to its oncogenic potential.

A. Individuals in Attendance:

1. Peer Review Committee: (Signatures indicate concurrence with the peer review unless otherwise stated.)

Penelope A. Fenner-Crisp	Penelope A. Fenner-Crisp
William L. Burnam	
Reto Engler	
Edwin R. Budd	Edwin R Budd
Marcia Van Gemert	
Karl Baetcke	
Marion Copley	
Kerry Dearfield	
Richard Levy	

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A. 1. Peer Review Committee (contd.)

John Quest _____

Esther Rinde Esther Rinde

William Sette William Sette

Lynnard Slaughter L. G. Slaughter

2. Reviewers: (Non-committee members responsible for data presentation; signatures indicate technical accuracy of panel report.)

Henry Spencer Henry Spencer

3. Peer Review Members in Absentia: (Committee members who were unable to attend the discussion; signatures indicate concurrence with the overall conclusions of the Committee.)

Richard Hill —

Robert Beliles _____

George Ghali G. Ghali

4. Other Attendees:

Esther Saito (HED) was also present.

B. Material Reviewed:

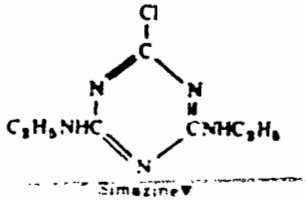
The material available for review consisted of DER's, one-liners, and other data summaries prepared by Dr. Henry Spencer; tables and statistical analysis by Dynamac. The material reviewed is attached to the file copy of this report.

C. Background Information:

Simazine is one of several triazine compounds which are used in agriculture as herbicides to control annual grasses and broadleaf weeds in corn, alfalfa, orchards of cherries, peaches, citrus, apples, pears and asparagus as well as ornamentals and nursery stock. Simazine is also registered for use in controlling algae in ponds. Little of the Simazine parent chemical is found as residues in food and feed crops.

Following the Data-Call-In Notice of the first Registration Standard of 1984, new chronic toxicity studies were received; these were evaluated by the Onco Peer Review Committee.

Structure of Simazine:



Simazine▼

D. Evaluation of Oncogenicity Evidence for Simazine:

1. CD-1 Mouse Oncogenicity Study

Reference: Hazelette, JR and JD Green: "Simazine Technical; 95-week Oral Toxicity/Oncogenicity Study in Mice.", April 4, 1988. Accession/MRID Number: 406144-04, Lab. Study Number: 842121. Testing Facility: Pharmaceuticals Division, Ciba-Geigy Corp., Summit, NJ.

Simazine technical was administered in the diet to groups of 60 male and 60 female Crl:CD1(ICR)BR mice at 0 (control), 40, 1000 or 4000 ppm for 95 weeks.

There were no increases in neoplasms reported for any dosed group.

There was no evidence of a compound-related effect on survival or target organ toxicity.

The dosing was considered to be adequate for assessing the oncogenic potential of Simazine, based on body weight gain depressions of 14% in males and 19% in females seen at 1000 ppm.

D. Evaluation of Oncogenicity Evidence (contd.)

2. Sprague-Dawley Rat Oncogenicity Study

Reference: McCormick, CC and AT Arthur: "Simazine-Technical: 104-Week Oral Chronic Toxicity and Carcinogenicity Study in Rats." , April 12, 1988. MRID Number: 406144-05. Study Number: 2-0011-09. Testing Facility: Pharmaceuticals Division, Ciba-Geigy Corp., Summit, NJ.

Simazine technical was administered in the diet to groups of 50 male and 50 female rats at 0 (control), 10, 100 or 1000 ppm for 2 years. Additional groups (30-40/sex/dose) were also treated.

> In female rats there was a statistically significant increase in mortality, and in male rats there was a statistically significant decrease in mortality, with increasing doses of Simazine.

Neoplastic lesions which occurred with statistically significant increases were reported as follows:

In female rats, there was a statistically significant dose-related trend ($p<.01$) for mammary gland carcinomas and combined adenomas/fibromas/carcinomas; however, when the shortened life-span of the female rats was included in the statistical evaluation, the incidences of carcinoma alone at both the 100 and 1000 ppm (HDT) dosage groups were statistically significantly increased as well ($p<.05$ and $p<.01$, respectively). The upper limit of the historical control incidence reported for mammary carcinoma (Table 1) was exceeded at 100 ppm, and greatly exceeded at 1000 ppm (HDT). The incidence of cystic glandular hyperplasia in the mammary gland was statistically significantly increased at the HDT, which correlates with the observed high tumor incidence at that dose.

There was a statistically significant dose-related trend for kidney tubule adenomas ($p<.05$); however (as in the case of the male rats) tumors occurred only at the HDT and the incidence (3.6%) was not statistically significant by pairwise comparison with that in the concurrent control. The incidences for adenomas and/or carcinomas reported for historical female controls (Table 1) were zero in all 7 studies (Table 1).

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TABLE 1
HISTORICAL CONTROL TUMORINCIDENCE DATA
NUMBER OF TUMOR-BEARING ANIMALS - SPRAGUE-DAWLEY RATS

Submitted by Ciba-Geigy

COMPOUND	83 A	JAN 83 B	NOV 83 C	84 D	85 E	85 F	85 G
SITE: NEOPLASM			NUMBER OF NEOPLASMS				
MAMMARY GLAND (FEMALES):							
NUMBER OF SITES EXAMINED	(65)	(60)	(70)	(70)	(60)	(70)	(70)
ADENOMA	6	6	8	2	5	3	2
FIBROADENOMA	18	16	26	21	12	23	22
ADENOMA/FIBROADENOMA (COMBINED)	22	18	30	22	15	25	23
ADENOCARCINOMA	7	4	5	11	9	15	14
ALL MAMMARY TUMORS (COMBINED)	25	22	34	30	20	34	32
PITUITARY GLAND (FEMALES):							
NUMBER OF SITES EXAMINED	(63)	(60)	(69)	(69)	(60)	(70)	(70)
ADENOMA	52	49	55	59	49	62	62
CARCINOMA	0	2	2	2	6	2	1
ADENOMA AND CARCINOMA (COMBINED)	52	51	57	61	55	64	63
KIDNEY (MALES AND FEMALES):							
NUMBER OF SITES EXAMINED	(65/65)	(60/59)	(70/70)	(70/70)	(60/60)	(70/70)	(70/70)
	M F	M F	M F	M F	M F	M F	M F
ADENOMA	0 0	0 0	2 0	1 0	0 0	0 0	0 0
CARCINOMA	0 0	0 0	0 0	1 0	0 0	0 0	0 0
ADENOMA AND CARCINOMA (COMBINED)	0 0	0 0	2 0	2 0	0 0	0 0	0 0
ADRENAL GLAND (FEMALES							
NUMBER OF SITES EXAMINED	(65)	(60)	(70)	(70)	(60)	(70)	(70)
ADENOMA	1	3	4	2	3	2	8
LIVER (MALES):							
NUMBER OF SITES EXAMINED	(65)	(60)	(70)	(70)	(60)	(70)	(70)
ADENOMA	0	2	0	2	10	4	1
CARCINOMA	0	1	1	6	2	1	0

50

50

D. Evaluation of Oncogenicity Evidence (contd.)

2. Sprague-Dawley Rat Oncogenicity Study (contd.)

In female rats, there were also statistically significant dose-related trends for adenomas, carcinomas and combined adenoma/carcinomas of the pituitary gland ($p<.01$). Pairwise comparisons were significant only for carcinomas at 1000 ppm ($p<.05$) and only when time adjusted, assuming fatal tumor context, to account for the effect of mortality disparity in the animals (the mortality in female rats was statistically significantly increased compared to controls at 100 and 1000 ppm). The incidence of pituitary gland carcinoma at 1000 ppm (HTD) only slightly exceeded the upper bound of the historical control range; however, it greatly exceeded the incidence reported in 6 out of 7 studies.

Tables 4, 5 and 6 (from the Dynamac "..Qualitative Risk Assessment...." 10/18/88, attached) summarize these findings; a fatal tumor analysis was performed on the female rat pituitary gland tumors, as described on pg. 8 of that memo.

Historical control tumor incidence data for Sprague-Dawley rats at the testing facility are given in Table 1.

In male rats, the incidences of liver tumors were statistically significantly increased for carcinoma and for combined adenoma/carcinoma at 100 ppm and 1000 ppm (HDT), respectively ($p<.05$); however, these incidences fell within the range reported for historical controls at the testing facility.

There was also a statistically significant dose-related trend for kidney tubule carcinomas ($p<.05$), and for combined adenoma/carcinoma ($p<.01$); however, tumors occurred only at the HDT and neither the carcinoma (3%) nor the combined adenoma/carcinoma (5%) incidence was statistically significant by pairwise comparison with that in the concurrent control (2% in both cases).

Tables 7 and 9 (from the attached Dynamac memo) present data for the tumor incidences (adjusted for mortality differences) in liver and kidney, respectively. The rationale for the tumor analysis is presented on page 8 of the Dynamac memo.

Table 4. 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	Historical Control Range (%)
Adenoma Fibroadenoma	23/89 (26)	20/78a (26)	11/71 (15)	21/75 (28)	(27-37)
	p= 0.3629	p= 0.302	p= 0.177	p= 0.123	
Carcinoma	16/89 (18)	13/80 (16)	20/75b (27)	40/78 (51)	(7-21)
	p< 0.0001**	p= 0.4740	p= 0.0392*	p< 0.0001**	
Adenoma Carcinoma	39/89 (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.

Table 5. SIMAZINE SPRAGUE-DAWLEY RAT Study-- Female Kidney Tubule Tumor Rates* and Cochran-Armitage Trend Test and Fisher's Exact Test

DOSE(PPM)	0.000	10.000	100.000	1000.000	Historical Controls
Adenoma	0/74 (0.0)	0/62 (0.0)	0/54 (0.0)	2/55c (3.6)	(all 0)
	p= 0.0042**	p= 1.0000	p= 1.0000	p= 0.1799	

c First Adenoma observed at 71 weeks in dose 1000 ppm. No carcinomas were coded.

\- Number of tumor bearing animals/Number of animals at risk (excluding animals that died before the observation of the first tumor or animal not examined).

( ) Per cent

Note: Significance of trend denoted at Control. Significance of pair-wise comparison with control denoted at 2sig level. * denotes p < 0.05 and ** denotes p < 0.01

TABLE 6. 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	Historical Control Range (%)
Adenoma	73/89	57/80	63/77 a	61/79	
	(82.0)	(71.2)	(81.8)	(77.2)	(80-89)
	p= 0.0013**	p= 0.9946	p= 0.0206*	p= 0.0030**	
Carcinoma	1/73	3/61	0/52	6/53 b	
	(1.4)	(4.9)	(0.0)	(11.3)	(0-10)
	p= 0.0010**	p= 0.2351	p= 0.4545	p= 0.0153*	
Adenoma Carcinoma	74/89	60/80	63/77	67/79	
	(83.1)	(75.0)	(81.8)	(84.8)	(83-92)
	p= 0.0005**	p= 0.8351	p= 0.0251*	p=0.0005**	

* Number of tumor bearing animals/Number of animals at risk (excluding animals that died before the first tumor or animals not examined).

( ) Per cent

a First Adenoma observed at 35 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 $p > 0.01$

Table 7. SIMAZINE SPRAGUE-DAWLEY RAT Study-- Male Liver Tumor Rates* and Cochran-Armitage Trend Test and Fisher's Exact Test Results

DOSE (PPM)	0.000	10.000	100.000	1000.000	Historical Control Range (%)
Adenoma	1/88	2/79a	0/80	3/80	
	(1.1)	(2.5)	(0.0)	(3.8)	(0-17)
	p= 0.0824	p= 0.4594	p= 0.5238	p= 0.2752	
Carcinoma	0/88	2/79	4/80b	3/80	
	(0.0)	(2.5)	(5.0)	(3.8)	(0-9)
	p= 0.2169	p= 0.2223	p= 0.0494*	p= 0.1058	
Adenoma or Carcinoma	1/88	4/79	4/80	6/80	
	(1.1)	(5.1)	(5.0)	(7.5)	
	p= 0.0643	p= 0.1519	p= 0.1554	p= 0.0449*	

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 in which male not examined).

Significant

Note: Significance of trend denoted at Control. Significance of pair-wise comparison with control denoted at Dose level. * denotes $p < 0.05$ and ** denotes $p < 0.01$

Table 9. 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	Historical Control Range
Adenoma	0/51 (0)	0/46 (0)	0/48 (0)	1/57a (2)	(0-3)
	p= 0.0543	p= 1.0000	p= 1.0000	p= 0.5278	
Carcinoma	1/66 (2)	0/62 (0)	0/64 (0)	2/65b (3)	(0-1)
	p= 0.0332*	p= 0.7660	p= 0.1821	p= 0.2091	
Adenoma Carcinoma	1/66 (2)	0/62 (0)	0/64 (0)	3/65 (5)	(0-3)
	p= 0.0056**	p= 0.1610	p= 0.1721	p= 0.1087	

a First Adenoma observed at 92 weeks in dose 1000 ppm.

b First Carcinoma observed at 73 weeks in dose 1000 ppm

c 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.

- 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).

( ) Per cent

Note: Significance of trend denoted at Control. Significance of pair-wise comparison with control denoted at dose level. * denotes $p < 0.05$ and ** denotes $p < 0.01$

D. Evaluation of Oncogenicity Evidence (contd.)

2. Sprague-Dawley Rat Oncogenicity Study (contd.)

The Committee agreed that the highest dose exceeded the MTD for female rats, based on excess deaths and body weight gain reductions of 28-45% (days 7-728). The highest dose in males appeared to have exceeded the MTD, as well, based on body weight gain reductions of 27-36% (days 7-728). The Committee also felt that there was too great an interval between the mid and high doses (100 to 1000 ppm).

E. Additional Toxicology Data on Simazine:

1. Metabolism

Simazine exhibits increased binding affinity for red blood cells following oral dosing in the rat. Almost all of orally administered Simazine was excreted in the feces and urine 96 hours after administration to rats.

2. Mutagenicity

Three mutagenicity tests have been submitted in support of the registration for Simazine. Simazine was negative in an acceptable Salmonella assay using strains TA98, TA100, TA1535, TA1537 and TA1538, with and without activation. The other two tests were found to be unacceptable: a cytogenetics assay with cultured human lymphocytes and an unscheduled DNA synthesis (UDS) assay with primary rat hepatocytes. Therefore, of the three categories of mutagenicity testing, only the gene mutation category is minimally fulfilled with data gaps in the structural chromosomal aberrations and other genotoxic effects categories.

The negative Salmonella results are consistent with published literature and results with other s-triazine herbicides. However, it is reported in the literature that Simazine is positive for gene mutations in the mouse lymphoma assay (Waters et al., Basic Life Sci 21: 275-326, 1982), the Drosophila sex-linked recessive lethal assay (ibid; also reported by the U.S. EPA Gene-Tox Program), cell transformation in Syrian hamster embryo cells (reported by the U.S. EPA Gene-Tox Program), and plant cytogenetic assays (for review see Plewa et al., Mutat Res: 136 233-245, 1984). Simazine was also reported in the literature as being negative in several other assays including yeast assays, UDS with a human cell strain, sister chromatid exchanges and a mouse micronucleus (an unacceptable protocol) (Waters et al. 1382). It was also reported negative in two assays for aneuploidy (see Dellarco et al., Mutat Res 167: 149-169, 1986 .

E. 2. Mutagenicity (contd.)

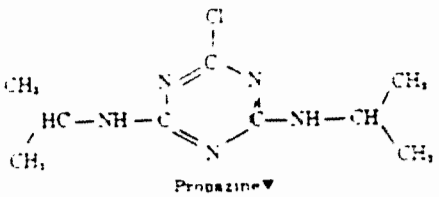
It appears then that Simazine has genotoxic potential and this would provide some support for an oncogenicity concern. Tests for submission to satisfy data gaps and to examine in more detail this genotoxic potential should include a mouse lymphoma assay, an in vivo micronucleus test and a cell transformation assay.

3. Developmental Toxicity

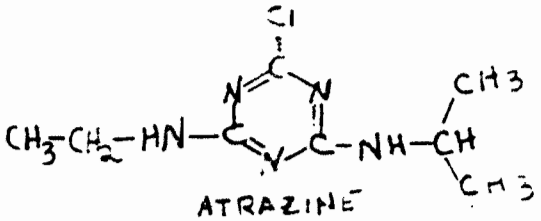
Simazine did not produce terata in the rat, when given by gavage at doses up to 600 mg/kg or in the rabbit at doses up to 200 mg/kg, by gavage; however, maternal toxicity and fetotoxicity (incomplete ossification) were observed in both species.

4. Structure-Activity Correlations

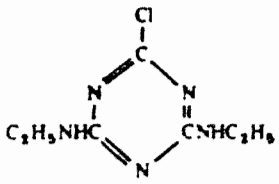
Simazine is structurally related to Atrazine, Propazine, Cyanazine, Ametryn and Prometryn. Atrazine was associated with increased mammary gland tumors in the female albino rat and was categorized as a "C(q)" oncogen by the HED Peer Review Committee. Propazine was also associated with increased mammary gland tumors in the female CD-1 rat and was categorized by the Committee as a "C" oncogen. Ametryn, Prometryn and Cyanazine have not yet been evaluated.



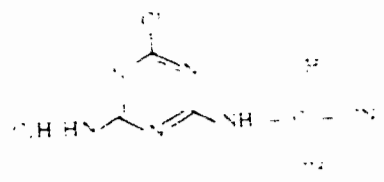
Propazine▼

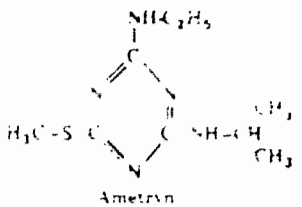


ATRAZINE



Simazine▼





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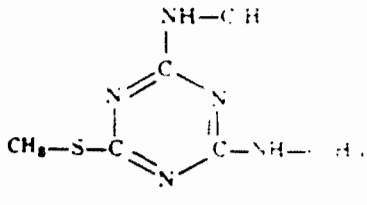

Prometryn

F. Weight of Evidence Considerations:

The Committee considered the following facts regarding the toxicology data on Simazine to be of importance in a weight-of-the-evidence determination of oncogenic potential.

1. Simazine was not associated with increases in neoplasms when fed in the diet to CD-1 mice, at doses up to 4000 ppm. The study was considered to have been adequately conducted.

2. Simazine was associated with statistically significant increases in carcinomas of the pituitary gland (at the HDT) and mammary gland (at the mid (100 ppm) and highest dose) in the female Sprague-Dawley rat, when fed in the diet at doses up to 1000 ppm. The incidence of mammary gland tumors at the HDT was well outside the range reported for historical controls at the testing facility. The incidence of pituitary gland tumors was just outside the historical control range; however, it exceeded (considerably) the incidences reported for 6 out of 7 studies.

3. The pituitary tumors in the female rats were fatal with a possibly accelerated onset, and the mammary carcinomas also contributed to the increased mortality at the HDT, according to the study authors.

4. Although the HDT may have exceeded the MTD, the mid-dose was well below, and the mammary tumors in the female rat were statistically significantly increased at both the mid and high dose. There was also too great an interval between the mid and high doses: 100 and 1000 ppm, respectively.

5. While a hormonal influence was suggested based on the pituitary and mammary gland tumors, supporting evidence was not presented.

6. There was some evidence of genctoxicity.

7. The mammary tumor response is consistent with that seen with other triazines. Both Atrazine and Propazine, triazines with structures closely related to Simazine, were associated with mammary gland tumors in the female rat.

F. Weight of Evidence (contd.)

8a. The incidence of kidney tubule adenomas at the HDT in the female rat, although not statistically significant, exceeded that reported for historical controls (zero) in all seven studies at the testing facility. While this tumor incidence fits the NTP definition of a "rare" tumor (≤1% incidence), Dr. Slaughter offered, that based on his experience, the historical incidence of rat kidney tumors is more accurately defined as "uncommon").

8b. The incidence of kidney tubule carcinomas in male rats was less clearly defined (because of sporadic occurrences of the same tumor in control animals).

G. Classification of Oncogenic Potential:

Criteria contained in the EPA Guidelines [FR51: 33992-34003, 1986] for classifying a carcinogen were considered.

The Committee evaluated all of the evidence listed in part F (above) and concluded that Simazine should be classified as a Category C Oncogen (possible human carcinogen), based on evidence in one species, one sex. The Committee also called for a quantitative risk assessment for Simazine, quantification to be based on the mammary tumors in the female rat. The arguments for quantification were given as follows:

1a. The tumors in both the pituitary and mammary glands of the female rat were malignant.

1b. Pituitary tumors in female rats were fatal with a possible accelerated onset (analysis to be provided).

2a. Mammary tumors were statistically increased at 2 doses, albeit one above the MTD; however, there was too large a spread between the mid and high doses.

2b. Evidence of progression was suggested by mammary hyperplasia at the HDT, which correlated with tumors at that dose.

3. There was no supporting evidence for demonstrating an hormonal influence.

4. There was equivocal evidence of kidney tumors ("rare" or at least "uncommon" tumor type) in both sexes.

5. SAR was strongly supportive. Other closely-related triazines (Atrazine and Propazine) were also associated with mammary gland tumors in the female rat.

6. There was some evidence of genotoxicity.

Quantitative Risk Assessment (Q_1*)

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UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
WASHINGTON, D C. 20460

OFFICE OF
PESTICIDES AND TOXIC SUBSTANCES

Subject: Simazine - Quantitative Risk Assessment, Two Year Chronic/Oncogennicity Sprague-Dawley Rat Study

caswell no. 740

From: Bernice Fisher, Biostatistician Bernice Fisher 6/5/89
Science Support Section
Science Analysis and Coordination Branch
Health Effects Division (H7509C)

To: Henry Spencer, Ph.D., Pharmacologist
Review Section II
Toxicology Branch I - Insecticide/Rodenticide Support
Health Effects Division (H7509C)

Thru: John A. Quest, Ph.D., Section Head John A Quest 6/5/89
Science Support Section
Science Analysis and Coordination Branch
Health Effects Division (H7509C)

Summary

The unit risk, Q_1^*, of simazine is 1.20×10^{-1} $(mg/kg/day)^{-1}$ in human equivalents. This estimate of Q_1^* is based upon mammary gland carcinomas in female Sprague-Dawley rats with dose levels of 0, 10, 100, and 1000 ppm.

Female rats had a significantly increasing trend in mortality with dose increments of simazine. There were significant differences in mortality in 2 dose groups, 100 and 1000 ppm as compared with controls. The females exhibited a significantly increasing trend in mammary gland carcinomas with increasing doses of simazine. In the pairwise comparison with controls, 2 dose levels, 100 and 1000 ppm, were also significantly different. See the memorandum on "Simazine - Qualitative Risk Assessment from a Rat Two Year Oral Chronic Toxicity and Oncogenicity Study, Dynamac (Dynamac no. 1-16, EPA: 68-D8-0565) - 10/18/88 for details.

Background

The Peer Review Committee on simazine on 5/17/89 concluded that the chemical compound should be classified as a [C_2] carcinogen. In addition they recommended that the unit risk, Q_1^*, should be estimated from the female rat mammary gland carcinoma tumor rates.

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Dose-Response Analysis

As a result of the Peer Review Committee's recommendation of the use of rat mammary gland carcinomas for the estimation of Q_1^* and since there was a significantly increasing trend in mortality in female rats with dose increments of simazine, the calculation of the unit risk was made by the use of Weibull83 (time-to-death with tumor multistage model by K.Crump) computer program. The unit risk calculated from the female data in ppm doses was converted to rat mg/kg/day by the use of Lehman's Tables and then to human equivalents by the use interspecies surface area adjustments as recommended by EPA Cancer Guidelines (1986).

The resultant estimate of Q_1^* is as follows:

	Rat, Q_1^* $(mg/kg/day)^{-1}$	In Human Equivalents
female mammary gland carcinoma tumors	2.25×10^{-2}	1.20×10^{-1}

It is to be noted that Q_1^* is an estimate of the upper (95%) bound on risk and that (as stated in the EPA Guidelines) the "true value of the risk is unknown and that the lower limit of the risk may be as low as zero".

References:

Krewski, D., Crump, K.S., Farmer, J., Gaylor, D.W., Howe, R., Portier, C., Salsburg, D., Sielken, R.L., Van Ryzin, J. (1983) A Comparison of Statistical Methods for Low Dose Extrapolation Utilizing Time-to-Tumor Data. Fundamental and Applied Toxicology 3: 140-160.

L. Data Evaluation Reports

81-1

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. 3/3/89
Section Head, Section II, Toxicology Branch I - IRS (TS-769C)

007240

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):

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.

81-2

00:240

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:

The 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.

67

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.

007240

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.

69

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.

81-4

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) 007240

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.

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71

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.

81-5

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 00724O
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.

81-6

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)

007240

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.

82-1

007240

Peviewed by : George Robinson, DVM
Secondary Reviewer: Robert Jaeger
Date: September 9, 1985

See Memorandum: ID # 100-541
Simazine Registration Standard
Recent Toxicity Studies.
Acc. Nos. 257692, 257693, 257694, 244268

Revaluated by Henry W. Spencer, Ph.D. 6/30/89
Secondary Reviewer: Marion P. Copely, DVM. 8/3/89

Data Evaluation Report

Chemical: Simazine

Toxicity Chemical No. 740

Purity: Technical, Batch No. FL 840968, a white powder 97.5%

Study Type: Subchronic, oral toxicity

MRID No. ~~000~~ 00143265

Acc. No. 257693

Sponsor: Ciba-Geigy Corp.

Testing Facility: Pharmaceuticals Division, Ciba-Geigy

Title of Report: Simazine Technical Subacute Oral 13-week Toxicity Study in Rats

Authors: C.N. Tai, C. Breckenridge and J. D. Green

Study No. Report No.85018

Report Issued: April 10, 1985

Conclusions:

The previous reviewers evaluation (copy attached) accurately reflects the results of the study. The study is classified as Core-Supplementary because a NOEL was not determined.

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Results: NOEL is less than 200 ppm: (LDT) reduction in erythrocyte (m & f) and leucocyte (m) counts: elevated cholesterol and inorganic phosphate levels (m & f): renal calculi in 3/20 rats (m & f).

MTD is less than 2000 ppm: seriously affected nutrition of treated rats (m & f).

Attachments 1
2
3

82-1

UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
WASHINGTON, D C 20460

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004655

SEP 12 1985

OFFICE OF PESTICIDES AND TOXIC SUBSTANCES

MEMORANDUM

SUBJECT: ID #100-541.
Simazine Registration Standard:
Recent Toxicity Studies.
Acc. Nos. 257692, 257693, 257694 and 244268
CASWELL #740

FROM: George W. Robinson, D.V.M. George W. Robinson 9/9/85
Review Section I
Toxicology Branch
Hazard Evaluation Division (TS-769C)

TO: Richard Mountfort, PM-23
Fungicide-Herbicide Branch
Registration Division (TS-767C)

THRU: Robert B. Jaeger, Section Head
Review Section I
Toxicology Branch
Hazard Evaluation Divison (TS-769C)

Registrant: Ciba-Geigy Corporation

The registrant has submitted several toxicity studies for review as a partial update of its toxicology data base required in accordance with EPA's Guidance Document for reregistration of simazine.

Results of toxicological reviews are as follows:

a. 13-Week Oral Feeding Study in Rats

NOEL < 200 ppm; reduction in erythrocyte (m & f) and leucocyte (m) counts; elevated cholesterol and inorganic phosphate levels (m & f); renal calculi in 3/20 rats (m & f).

MTD < 2000 ppm; seriously affected nutrition of treated rats (m & f).

Classification: Core-Supplementary Data

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b. 13-Week Oral Feeding Study in dogs

NOEL = 200 ppm; reduced albumin levels and increased globulin levels (m), and elevated urinary specific gravity (m) and ketone levels (m & f) at 2000 ppm.

MTD < 2000 ppm; seriously affected nutrition of treated dogs (m & f).

Classification: Core-Minimum Data.

c. Metabolism of Simazine in Female Rats

1. The numbers of animals/sex, animal groups and dose levels were too few. Only 2 female rats received single doses of ^{14}C-simazine; and,

2. Measurement intervals and observation period were inadequate.

Classification: Core-Supplementary Data.

d. Acute Inhalation (4 hours), rats

LC_{50} > 2.1 mg/liter

Classification: Core-Minimum Data; Category III.

Attachment 2

004656
00240

1. Simazine Technical Subacute Oral 13-Week Toxicity Study in Rats by C.N. Tai, C. Breckenridge and J.D. Green, Pharmaceuticals Division, Ciba-Geigy Corporation, Report No. 85018, April 10, 1985; Acc. No. 257693.

Test Material:

Simazine Technical, Batch No.: FL 840988, white powder, purity of 97.5%.

Test Animals:

Male and female rats Sprague-Dawley [Crl: COB® CD® (SD) BR] from Charles River Breeding Laboratories, Kingston, N.Y. Rats were caged individually under standard controlled laboratory conditions with feed and water ad libitum. Rats were 7 weeks old when received and 10 weeks at initiation of the study.

Experimental Design:

Rats were randomly assigned to one of 4 groups of 10/sex and were fed simazine in a powdered feed admixture ad libitum at concentrations of 0, 200, 2000 and 4000 ppm, respectively. Homogenous blends of Simazine Technical in the powdered feed diet were prepared weekly and fed to the rats for 13 consecutive weeks (7 days a week). Rats in the control group received simazine-free diet.

Physical and ocular examinations were conducted on all rats prior to initiation of the study. All animals under went weekly physical examinations which included palpation for tissue masses. Each rat was observed twice daily for mortality and signs of toxicity weekdays and once daily weekends and holidays. Pre-dose body weights and weekly body weights and food consumption were recorded.

Ten tested rats sex from original stock were bled to determine baseline hematology and clinical chemistry values. Blood was also collected from all surviving rats just prior to termination for determination of terminal hematology and clinical chemistry values. Urine samples were collected from surviving rats prior to termination for routine urinalysis.

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All surviving rats were sacrificed and necropsied at the end of the dosing period (91 days). All gross lesions and tissue masses as well as adrenal gland, aorta, sternal bone and marrow, brain, cecum, colon, duodenum, esophagus, epididymis, eye, optic nerve, femoral bone marrow, gonad, heart, ileum, jejunum, kidney, larynx/pharynx, liver, lung, submaxillary and mesenteric lymph node, mammary gland, skeletal muscle, pancreas, parathyroid gland, prostate, rectum, salivary gland, pituitary gland, seminal vesicle, sciatic nerve, skin, spinal cord, spleen, stomach, tongue, thymus, thyroid gland, trachea, urinary bladder, uterine cervix and horn, and vagina were collected from all rats (as applicable to sex) and fixed in 10% neutral buffered formalin. Fixed tissues of control and high dose groups underwent routine histologic processing, were stained with H&E, and were examined microscopically. Adrenal gland, heart, kidney, liver, lung and spleen from low- and mid-dose rats were processed similarly and examined microscopically.

Results:

Rats at all dose levels survived the 13-week feeding study in good physical condition with no apparent treatment-related clinical signs. There were no ocular changes observed.

The calculated mean daily doses of Simazine decreased during the 13-week period with ranges in low, mid and high dose groups as follows: 9.6 to 17.4, 104.6 to 151.8 and 199.3 to 289.7 mg/kg in males and 13.3 to 18.8, 143.3 to 186.4 and 234.2 to 343 mg/kg in females.

Significant dose-related reductions in mean feed intake were observed during the first week of dosing in males and females, respectively: 4000 ppm (52.5% & 46.4%); 2000 ppm (37.2% & 33.1%) and, 200 ppm (6.5% & 7.9). Feed intake increased during the 2nd week for males and females in all treated groups, but mean feed intake of mid- and high-dose rats remained significantly less than control rats throughout the dosing period. Mean feed intake in low- dose females was similar to that in control females during weeks 2 through 13. Low-dose males, however, had significantly reduced mean feed intake compared to control males during weeks 5 through 11.

Concomitantly, significant dose-related reductions in mean body weights and mean body weight gains occurred in males and females in all treated groups. Significant weight loss occurred in mid- and high-dose males and females during the first week of dosing. During weeks 2 through 13, significantly reduced mean body weights and mean body weight gains were observed in treated males at all dose levels when compared to control males. Mean body weights and body weight gains were significantly less in mid- and high-dose females during weeks 1 through 13. Although reduced mean body weight gains were recorded, mean body weights of low-dose females were not significantly different from those of control females.

At 13 weeks dose-related reductions in mean erythrocyte counts were detected in all treated rats (both sexes) with accompanying decreased hematocrit levels in females at mid- and high-dose levels and in males at the high-dose level. Mean leukocyte counts were significantly lower in males at all dose levels. Neutrophil and platelet counts were significantly higher in female rats at mid- and high-dose levels, with a dose-related increase in males (not significant) at all doses.

A variety of significant differences from control rats were detected in clinical chemistry and urinalysis determinations in all dose groups but were most marked in mid- and high-dose groups. Significantly lowered mean blood glucose levels occurred in male rats. High cholesterol and inorganic phosphorus levels were present in males and females. Other differences included low levels of sodium and calcium in males, low levels of BUN, LDH, SGOT and creatinine in females, elavated urinary ketone levels in males, and decreased urinary protein levels in females.

Reduced absolute organ weights and increased relative organ weights for brain, heart, kidney, liver and testes in males and for brain and spleen in females were recorded for mid- and high-dose groups. Increased mean relative adrenal weights occurred in both sexes in all dose groups. Reduced mean absolute spleen weight in males and mean absolute ovary and heart weights were observed in mid- and high-dose groups without appreciably increasing relative organ weights. Relative ovarian weight was actually reduced in high-dose females.

Necropsies revealed no gross lesions attributable to the feeding of Simazine at 200, 2000 and 4000 ppm. A dose-related incidence of renal calculi were detected in treated rats when kidneys were examined microscopically.

	Number of renal calculi at doses (ppm) of			
Sex	0	200	2000	4000
Males	0/10	1/10	3/10	5/10
Females	1/10	2/10	5/10	2/10
Total	1/20	3/20	8/20	7/20

The incidence of calculi was significant for males in the high-dose group and for males and females combined in the mid- and high-dose groups. The incidence of renal epithelial hyperplasia, tissue reaction to calculi, in high-dose males was also significant. The calculi were located primarily in the renal pelvic lumen, rarely in tubules. Microscopic examinations revealed no other lesions which could be attributable to a toxic effect of Simazine.

Conclusion:

It appears that reduced mean feed intake in treated rats is most likely due to the palatability of Simazine in the diet. Lower individual body weights and reduced body weight gains paralleled mean feed intake in treated rats. The majority of the alterations in clinical chemistry values may be related to feed consumption in treated rats. Renal calculi and attending hyperplasia were the only dose-related lesions detected microscopically.

NOEL < 200 ppm; reduction in erythrocyte (m & f) and leukocyte (m) counts; elevated cholesterol and inorganic phosphate levels (m & f); renal calculi in 3/20 rats (m & f)

MTD < 2000 ppm; seriously affected nutrition of treated rats (m & f)

Classification: Core-Supplementary Data

NOTE: A no-observed-effect-level was not determined.

82-1

Reviewed by George Robinson, DVM.
Secondary Reviewer: Robert Jaeger
Date: September 9, 1985

See Memorandum: ID# 100-541
Simazine Registration Standard
Recent Toxicity Studies
Acc. Nos. 257692, 257693, 257694, 244268

Reevaluated by: Henry W. Spencer, Ph.D. 6/30/89
Secondary Reviewer: Marion P. Copely, DVM 7/6/89

Data Evaluation Report

Chemical : Simazine

Toxicity Chemical No. 740

Purity: Patch No. FL 840988, Simazine Technical, 97.5% purity

Study Type: Subacute Oral Toxicity in Dogs

MRID No. - 00146655

Acc. No. 257692

Sponsor: Ciba-Geigy Corp.

Testing Facility: Pharmaceuticals Division, Ciba-Geigy

Title of Report: Subacute Oral 13-week Toxicity Study in [illegible]

Authors: C. N. Tai, C. Breckenridge and J. C. Green

Study No. 25022

Report Issued: April 12, 1985

Conclusion:
The previous reviewers evaluation (copy attached) accurately reflects the results of the study. The study is classified as core-minimum.

Results: After receiving 13 weeks of technical Simazine in diets of 0, 200, 2000 or 4000 ppm in the feed over a period of 13 weeks, Blood counts and clinical chemistry determinations did not indicate a toxic effect from the chemical. Tremors in both sexes were noted in the high dose only. Feed consumption was reduced in both mid and high dose animals. Mid and high dose females lost weight over the course of the study while only the high dosed males actually lost weight. The mid dosesd males also were affected by showing only a scant weight gain over the length of the study.

83
85

It appears that reduced mean feed intake in treated dogs is most likely, due to the palatability of Simazine in the diet. Lower individual body weights and reduced body weight gains paralleled mean feed intake in treated dogs. The majority of the alterations in clinical chemistry values and organ weights may be related to feed consumption in treated dogs.

NOFL = 200 ppm: reduced albumin levels and increased globulin levels (m), and elevated urinary specific gravity (m) and ketone levels (m & f)at 2000 ppm.

MTD is less than the 2000 ppm: seriously affected nutrition of the treated dogs (m & f).

82-1
00465
007240

2. Simazine Technical Subacute Oral 13-Week Toxicity Study in Dogs by C. N. Tai, C. Breckenridge and J. D. Green, Pharmaceuticals Division, Ciba-Geigy Corporation, Report No. 85022, April 12, 1985; Acc. No. 257692.

Test Material:

Simazine Technical (Batch FL 840988), purity of 97.5%; prepared weekly as an admixture of Simazine Technical in powdered Purina canine diet.

Test Animals:

Purebred Beagle dogs from Marshall Farms. Dogs were caged inidividually under standard controlled laboratory conditions with feed and water ad libitum. Dogs were approximately 7 to 8 months of age at the initiaiton of dosing and had a body weight range from 8.0 to 10.0 kg for males and 7.0 to 8.6 kg for females.

Experimental Design:

Dogs were assigned to one of 4 groups of 4/sex and were fed a dietary admixture of Simazine ad libitum at concentrations of 0, 200, 2000 and 4000 ppm, respectively. Control groups received non-treated Purina canine diet. Approximately 400 gram portions of the test feed admixtures and control diet were offered daily to dogs for at least 91 consecutive days.

Preliminary physical examinations of all dogs were conducted by the attending veterinarian. Only dogs judged healtny based on general observations, body weights, and on physical, clinical laboratory and ophthalmological examinations were chosen for this study. All dogs were observed daily for appearance, mortality and signs of toxicity. Further physical examinations of dogs were conducted on days 29, 57 and 91 of the study. Individual body weights and feed consumption were recorded predose and weekly thereafter; body weights were also recorded just prior to necropsy. Blood was collected from all dogs predose and at 44 and 92 days for hematologic and clinical chemistry determinations. Urine was collected from all dogs predose and at 42 and 93 days for routine urinalysis. Ophthalmological examinations were conducted on all dogs on day 91.

All dogs were sacrificed and necropsied between 93 and 98 days of the study with one exception; a low dose male bit a technician and was quarantined by law for 15 days and was sacrificed on study day 108. Specimens of all gross lesions and tissue masses, adrenal gland, aorta, sternal bone and marrow, brain, cecum, colon, duodenum, esophagus, epididymis, eye, gonads, gall bladder, heart, ileum, jejunum, kidney, liver, lungs, axillary and mesenteric lymph nodes, mammary gland, skeletal muscle, sciatic nerve, optic nerve, pancreas, parathyroid gland, pituitary gland, prostate, rectum, salivary gland, skin, spinal cord, spleen, stomach, tongue, thymus, thyroid gland, trachea, urinary bladder, uterine cervix and horn, and vagina were collected from all dogs (as applicable to sex) and fixed in 10% neutral buffered formalin. Fixed tissues underwent routine histologic processing, were stained with H & E, and were examined microscopically.

Results:

Dogs at all dose levels survived the 13-week feeding study. The mean daily intake of Simazine by dogs in the low, mid, and high dose groups was calculated to be as follows: 6.9, 65.2, and 133.6 mg/kg for males and 8.2, 64.3, and 136.7 mg/kg for females, respectively. Tremors, which were observed as early as week 3 and persisted until termination, occurred in 4/4 males and 3/4 females at the high-dose level only.

Mean daily feed consumption was significantly less in mid and high dose males and females than in control dogs throughout the 13-week dosing period. Compared to controls, mean feed consumption was reduced in mid and high dose groups as follows: 22.2 and 25.2% for males; and 32.0 and 35.9% for females. Mean feed consumption in low dose males and females did not differ significantly from that of controls.

High dose males actually lost body weight during the 13-week feeding study, -0.8 kg (-8.9%); mid dose males gained a scantly 0.1 kg (+ 1.14%) during the same period. Mid and high dose females lost 0.6 kg (-7.8%) and 1.4 kg (-18.7%) body weight, respectively.

Mean heart rate of high dose females was significantly higher than that of controls during study week 9; the same occurred in high dose males during study week 13. Also, mean body temperature was slightly higher in high dose males than in controls. Mean erythrocyte counts, hemoglobin and hematocrit values were significantly lower in high dose males and females than in controls during weeks 7 and 13. Mean percent neutrophils were significantly higher and mean percent lymphocytes were significantly reduced in high dose males at week 13. Mean platelet count was significantly high in high dose males during weeks 7 and 13. Mean percent eosinophils were significantly reduced in all treated males at week 7 and in high dose males at week 13. Mean prothrombin time was significantly less in all treated females relative to controls at week 13.

A variety of significant differences from control dogs were detected in various parameters in clinical chemistry and urinalysis determinations in all dose groups but were most marked in mid and high dose groups. Significantly lowered mean albumin levels occurred in mid and high dose males at week 13 accompanied by an increase in mean globulin levels and a decrease in A/G ratio in high dose males. There were also significant reductions of mean SGOT levels in all treated males at week 7 and in high dose males at week 13, in mean creatinine levels in high dose males at week 13, in mean total bilirubin in high dose males at week 7, and in Ca^{++} in high dose males at week 7 and mid and high dose males at week 13. Significant reductions occurred in mean alkaline phosphatase in mid and high dose females at week 7, in Ca^{++} levels in high dose females at week 13, and in Na^{+} and SGOT levels in mid and high dose females at week 13. Routine urinalysis revealed significant increases in specific gravity in mid and high dose males at week 7, and in ketone levels in mid and high dose males and females at week 7 and high dose males at week 13. Urinary pH was significantly decreased in high dose males at week 7 and high dose males and females at week 13.

Significantly reduced mean absolute heart weights were recorded for all treated males and high dose females. Mean relative heart weights were also significantly reduced in high dose males and females. Significantly higher mean relative brain and liver weights were recorded for mid and

high dose males and high dose females. Significantly higher mean relative brain and liver weights were recorded for mid and high dose males and high dose females. Mean absolute testes weights were significantly reduced in mid and high dose males; mean relative testes weight was also reduced in high dose males.

Necropsies revealed no gross or microscopic lesions attributable to the feeding of Simazine at 200, 2000 and 4000 ppm.

Conclusion:

It appears that reduced mean feed intake in treated dogs is most likely, due to the palatability of Simazine in the diet. Lower individual body weights and reduced body weight gains paralleled mean feed intake in treated dogs. The majority of the alterations in clinical chemistry values and organ weights may be related to feed consumption in treated dogs.

NOEL = 200 ppm; reduced albumin levels and increased globulin levels (m), and elevated urinary specific gravity (m) and ketone levels (m & f) at 2000 ppm.

MTD = 2000 ppm; seriously affected nutrition of treated dogs (m & f)

Classification: Core - Minimum Data.

TS-769:ROBINSON:sll:X73710:8 29/85 Card 7

82-2

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Reviewed by George Robinson, DVM.
Secondary Reviewer: Robert Jaeger
Date: September 9, 1985

See Memorandum: Simazine Registration Standarrd
11/4/83
Toxicology Chapter

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

Data Evaluation Report

Chemical : Simazine

Toxicity Chemical No. 740

Purity: Simazine Technical, 97.6% purity

Study Type: Subacute Dermal Toxicity

MRID No. 00005767

Acc. No. -

Sponsor: Ciba-Geigy Corp.

Testing Facility: Bio-Research Laboratories, Ltd., Canada

Title of Report: 21-Day Subacute Dermal Toxicity in Rabbits

Authors: Not given

Study No. 12017

Report Issued: April 14, 1980

Conclusion:
The previous reviewers evaluation (copy attached) accurately reflects the results of the study. The study is classified as core-guideline.

Results: After receiving 15 doses of technical Simazine in dose of 0, 10, 100, or 1000 mg/kg on the skin over a period of 21 days, Blood counts and clinical chemistry determinations did not indicate a toxic effect from the chemical via the dermal route.
A small number of animals (3/80) exhibited ulcerative dermatitis which was considered to be from technician handling. The NOEL was greater than 1000 mg/kg/day.
The registration requirement for this study is fulfilled.

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

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004255

OFFICE OF
PESTICIDES AND TOXIC SUBSTANCES

MEMORANDUM

TO: Richard Mountfort, PM #23
Registration Division (TS-767)

THRU: Robert B. Jaeger, Section Head RBJ 11/4/83
Review Section #1
Toxicology Branch/HED (TS-769)

SUBJECT: Simazine Registration Standard

Submission of the Toxicology Branch evaluation of Simazine toxicity data consists of:

1. Reviews of previously unreviewed studies.

2. Data Evaluation Reports for each relevant toxicity study.

3. "One-Liners" for the Data Base.

4. Data Summary, a bibliography indicating the toxicological data gaps and measures taken to fill them.

5. Policy discussion and tolerance assessment.

Toxicology Chapter

Acute Testing:

There are no available acute studies on the technical grade of the active ingredient. Acute studies submitted in MRID #00023965 were previously reviewed and Core classified by C. Frick (memo, 9/20/77 and TOX Doc. #001392). Simazine is a chlorotriazine herbicide and algaecide which has low toxicity from acute exposure.

93

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Subchronic Testing

82-2 21-Day Dermal

Study Type: 21-Day Subacute Dermal Toxicity in Rabbits.

MRID Number: 00057567

Sponsor: Ciba-Geigy Corp.

Contracting Lab: Bio-Resarch Laboratories, Ltd., Canada
Project No. 12017 Dated: 1/14/80

Test Material: Simazine Technical, 97.6% purity

Methods and Experimental Design:

New Zealand White rabbits from Canadian Breeding Farms and Laboratories were individually housed in cages under temperature-controlled conditions and acclimatized for two weeks. Eighty rabbits were equally divided into 4 test groups of 10 males and 10 females to receive Simazine Technical at doses of 0, 10, 100 and 1000 mg/kg. All animals were shaved 24 hours prior to initial testing and at necessary intervals thereafter. In addition, the skin of 5 rabbits/sex/dose were abraded and again once each week. All animals were weighed once prior to the initial application and twice weekly thereafter with dosing volume adjusted to the most recent body weight. Test material was weighed out in appropriate volumes on each day of dosing, slightly moistened with physiological saline and topically applied to intact and abraded skin sites with occlusive wrapping (10% body surface area). The control group was administered physiological saline concurrently. Applications were maintained for a period of six hours at each dosing after which the impervious wrap was removed and the skin wiped to remove any residual test material. Each animal received applications 5 days a week for a total of 15 applications over a period of 21 days. Viability and toxicity checks were made once each morning and late afternoon. Scoring of erythema and/or edema according to the technique of Draize was made daily. Estimates of food consumption were made weekly during the study.

Blood samples for biochemical and hematological analyses were withdrawn on days 0 and 21. The following were performed on each blood sample:

1. Complete blood count consisting of hemoglobin, hematocrit, WBC with differential, RBC, platelet and reticulocyte count, and

2. Analyses for BUN, glucose, alkaline phosphatase activity, S.G.P.T., S.G.O.T., calcium, potassium, lactic dehydrogenase, direct and total bilirubin, total cholesterol, total protein, albumin, globulin and A/G ratio.

On day 21 of the study each rabbit was sacrificed and immediately subjected to a complete gross necropsy. In addition, the liver, kidneys, heart, gonads, thyroid (with parathyroid), adrenals, and pituitary were examined and weighed.

Histopathological examination was performed on multiple sections of treated and untreated skin, on any gross lesion present (along with normal contiguous tissue), on representative samples of liver, kidney, brain (3 levels, from cerebellum, cerebrum and pons), heart, pituitary, thyroid with parathyroid, adrenals and gonads.

Results:

Dermal application of simazine technical to abraded and intact skin sites did not appear to produce any dose-related systemic toxicity in rabbits. Nonspecific intermittent episodes of lacrimation and pulmonary congestion occurred in control and treated groups. One high dose female died on day 11 of the study and gross necropsy revealed generalized congestion in most visceral organs.

Slight erythema was seen on the [illegible] of one high dose male during the first [illegible] study. One low dose female exhibited very slight erythema on 2 nonconsecutive days on intact skin. Signs of erythema and edema were absent in all other rabbits throughout the study. Ulcerative dermatitis which [illegible] test sites occurred in 2 mid [illegible] female.

Incidental differences occurred in both pre-treatment and post-treatment hematology and blood chemistry analyses. No significant differences were revealed between test groups. Miscellaneous subclinical lesions were observed at necrospy in control and test groups which were unrelated to simazine dosage.

Conclusion:

Fifteen dermal applications of technical simazine over 21 days at doses up to 1 g/kg produced no systemic toxicity nor any dose-realted alterations of the skin. Ulcerative dermatitis observed in 3/80 rabbits was most likely due to technical handling. NOEL > 1000 mg/kg

Classification: Core-guidelines

This study satisfies the registration requirement.

83-1
83-2

Reviewed By: Y.M. Ioannou YMI 10/26/88
Section II, Toxicology Branch I - IRS (TS-769C)
Secondary Reviewer: M. Copley M. Copley 11/4/88
Section II, Toxicology Branch I - IRS (TS-769C)

007240

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

07
96

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, 50 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[b]	
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.

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 40 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)	Dietary Concentration 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 - 119.40
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	372.3 (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	-1.8	-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)

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	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* (8.?)	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)	133.5 (1.9)	141.5** (2.1)	122.2** (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)	137.0* (1.6)
532	188.9 (3.3)	178.5 (4.4)	184.5 (3.2)	164.2** (2.7)	149.4 (3.0)	136.1 (4.9)	143.3 (4.9)	132.4* (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)	110.0 (10.5)	151.8 (11.1)

[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 significantly 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** (0.8
	537	43.5 (0.5)	42.6 (0.5)	42.8 (1.5)	37.9** (0.9)	44.0 (0.6)	41.2* (0.8
	725	41.4 (1.4)	41.2 (0.7)	36.4* (1.4)	34.3** (1.5)		
MCHB (mmicro gm)	361	22.3 (0.3)	22.7 (0.3)	23.3 (0.3)	23.7* (0.4)		
	537	21.3 (0.2)	21.6 (0.4)	21.8 (0.2)	22.8** (0.4)		
	725	22.6 (0.5)	22.0 (0.3)	22.8 (6.5)	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)	7.3 (0.6)
	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 0	10	100	1000	Recovery Group 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)				
	36	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	1.2
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.

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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

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.

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
Testis - 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 8
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	3/70	1/70	1/70	10/70*
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:

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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; ** $p < 0.01$

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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	0/24	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	2/70	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 C.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

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Dose (ppm)	0.000	10.000	100.000	1000.000
Adenoma Fibroadenoma	23/89 (26)	20/78a (26)	11/71 (15)	21/75 (28)
	p = 0.0689	p = 0.302	p = 0.177	p = 0.123
Carcinoma	16/89 (18)	13/80 (16)	20/75b (27)	40/78 (51)
	p < 0.0001**	p = 0.4740	p = 0.0392*	p < 0.0001**
Adenoma Carcinoma	39/89 (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 18 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), 1/10 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.

b. 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 is 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)	57/80 (71.2)	63/77[a] (81.8)	61/79 (77.2)
	p = 0.0033**	p = 0.0344	p = 0.0206*	p = 0.0100**
Carcinoma	1/73 (1.4)	3/61 (4.9)	0/52 (0.0)	6/58 (10.3)
	p = 0.0010**	p = 0.2351	p = 0.4545	p = 0.0153*
Adenoma Carcinoma	74/89 (83.1)	60/80 (75.0)	63/77 (81.8)	67/79 (84.8)
	p = 0.0005**	p = 0.8351	p = 0.0251*	p = 0.0006**

+ Number of tumor bearing animals/Number of animals at risk (excluding animals not examined).
() Percent
[a] First adenoma observed at 35 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)	0/54 (0.0)	2/55[c] (3.6)
	p = 0.0042**	p = 1.0000	p = 1.0000	p = 0.1799

[c]First adenoma observed at 71 weeks in dose 1000 ppm. No carcinomas were coded.
-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 = 0.1252
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 = 0.1159
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 = 0.0449*

[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 (2/79) and nonexistent in the treated groups (0/79, 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 = 0.0870
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	3/51 (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 heterogeneous 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.

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50890:I:Ioannou:C.Disk:KENCO:8/8/88:SG: :
R:50894:Ioannou:C.Disk:KENCO:10/11/88:rw:vo:ek:rw

83-1

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

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DATA EVALUATION REPORT

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

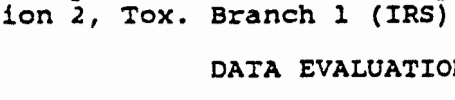
TOX. CHEM. No.: 740

MRID No.: 406144-02.

TEST MATERIAL: Simazine, tech.

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

STRUCTURE:



SPONSOR: Agrioultural 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.

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.

C. 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

(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:

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. %	Platelet 10**3/C
Controls	Pre-dose	5.97	14.8	44.5	1.7	336	6.33	15.0	44.8	1.1	277
	86	6.51	15.8	45.0	1.0	369	7.28	17.1	49.5	1.1	338
	177	6.32	15.1	44.0	0.5	253	7.24	17.1	49.2	0.9	269
	359	6.54	15.9	46.2	1.0	268	7.00	17.0	49.2	1.2	318
MDT	Pre-dose	5.64	13.6	41.3	2.0	350	6.20	15.3	45.2	0.8	232
	86	6.51	15.4	44.3	-	356	6.29	15.2*	43.5*	-	371
	177	6.79	16.4	46.8	-	257	6.48	15.5	45.0	-	289
	359	7.30	18.4*	51.8	-	246	6.82	17.0	48.8	-	341
HDT	Pre-dose	5.90	14.0	42.0	1.9	360	6.10	15.3	45.0	1.6	317
	86	5.80	13.9	38.8*	1.2	487*	6.04*	14.9*	42.5**	1.5	413
	177	6.18	14.9	42.5	0.8	432**	6.14*	15.0*	43.2*	1.4	370
	359	6.82	16.9	48.2	1.7	416**	6.30	16.0	46.0	1.6	369

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

14/0

Simazine RIN: 0569-93

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Pages 141 through 149 are not included.

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____ Identity of product inert ingredients.

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____ Identity of the source of product ingredients.

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____ A draft product label.

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

DATA EVALUATION RECORD

SIMAZINE

Chronic Toxicity/Oncogenicity Feeding Study in Mice

APPROVED BY:

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

Signature: Robert J. Weir

Date: 2-3-89

007240

EPA: 68D80056
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February 3, 1989

DATA EVALUATION RECORD

SIMAZINE

Chronic Toxicity/Oncogenicity Feeding Study in Mice

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007240

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.

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:

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 (HDT)	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(62)
4000	90	25	5(93)	17(72)	36(42)

[a]Percent survival was based on 80, 71, 70, and 80 males and 80, 71, 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	41.0 ± 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 ± 0.66
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**	36.0 ± 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 ± 0.88
40	20.3 ± 0.20	21.8 ± 0.18	32.4 ± 0.39	36.6 ± 0.64	36.9 ± 0.65	37.1 ± 0.98
1000	20.2 ± 0.18	21.5 ± 0.17	30.2 ± 0.27**	33.7 ± 0.42**	34.2 ± 0.50**	34.4 ± 0.60**
4000	20.5 ± 0.17	20.8 ± 0.16**	27.9 ± 0.22**	29.2 ± 0.32**	30.4 ± 0.41**	30.0 ± 0.52**

*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 652.1 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	364	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 ± 1.0
40	48.9 ± 0.86	48.0 ± 0.56*	43.3 ± 0.82	32.8 ± 0.44	28.2 ± 0.45	32.4 ± 1.0
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 ± 0.98
40	46.5 ± 0.80	44.9 ± 0.70	46.9 ± 0.90	34.1 ± 0.77	28.7 ± 0.51	32.2 ± 0.62
1000	47.4 ± 0.97	43.8 ± 0.79	44.3 ± 0.86*	32.2 ± 0.78	28.1 ± 0.61	32.6 ± 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 ± 0.92*

*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 ± 5.3
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 ± 6.4
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 ± 2.8
4000	28.5 ± 1.5*	23.4 ± 1.1**	21.7 ± 2.0**	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.

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:

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.

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 (10^6/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 ± 0.30
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 (10^6/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 $p < 0.01$.

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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		Glandular
X	Ileum	X	Eyes (optic nerve)	FXX	Adrenals
X	Cecum		Urogenital		Lacrimal gland
X	Colon	FXX	Kidneys	X	Mammary gland
X	Rectum	X	Urinary bladder	FXX	Thyroids/ parathyroids
XX	Liver	FXX	Testes		Harderian glands
X	Gallbladder	X	Epididymides		
X	Pancreas	X	Prostate		Other
		X	Seminal vesicle	X	Bone (sternum)
	Respiratory	FXX	Ovaries	X	Skeletal muscle
X	Trachea	XX	Uterus	X	Skin
XX	Lung	X	Vagina	X	All gross lesions and masses
X	Larynx/pharynx				

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.

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.

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	1.84 ± 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

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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)	(64)	(57)	(53)	(66)	(64)	(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	6	4	8*
Spleen	(70)	(70)	(70)	(70)	(70)	(69)	(69)	(71)
Amyloid	7	14	11	11	4	11*	5	4
Hyperplasia	9	6	3	12	6	5	8	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

TABLE 11. Neoplastic Findings in Mice Fed Simazine Technical for 94 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: 007240

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.

8.3-3

Primary reviewer: David G Anderson, PhD. David G Anderson 10/31/88
Section 2, Tox. Branch (IRS)(TS-769C).
Secondary reviewer: Marion Copley, DVM. Marion Copley 11/4/88
Section 2, Tox. Branch (IRS)(TS-769C).

007240

DATA EVALUATION REPORT

STUDY TYPE: Teratology/Rat/Simazine/822099.

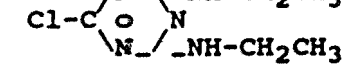
TOX. CHEM. No.: 740

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

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

A/D Ratio = 1.

00240

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: NOT 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
1. Cont.	2% methyl-cellulose 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.

5. Food and Water: - The food was a commercial diet, Purina #5002 Certified Chow. The water used was not specified. Both food and water were supplied ad libitum. 007240

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 copied 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.

Table A. 007240

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.

007240

	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.

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 RIN: 0569-93

Page____ is not included in this copy.

Pages 179 through 189 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.

✓ 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.

Attachment #3

CASWELL FILE

83-3

007240

005127

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

MAY 17 1986

MEMORANDUM

OFFICE OF
PESTICIDES AND TOXIC SUBSTANCES

SUBJECT: EPA Reg. No. 100-541, Simazine Technical: Ciba-Geigy Response to EPA Comments on Rabbit Teratology Study (Acc. #252938).
Acc. No. 260651 MRID No. 00161407 CASWELL #740

TO: Richard F. Mountfort, PM #23
Herbicide/Fungicide Branch
Registration Division (TS-767C)

FROM: George W. Robinson, D.V.M. George W. Robinson 5/8/86
Review Section #1
Toxicology Branch/HED (TS-769)

THUR: David L. Ritter, Acting Section Head DLR 5-9-86
Review Section I
Toxicology Branch/HED (TS-769C)

Registrant: Ciba-Geigy Corporation
Greensboro, NC

5/17/86

A rabbit teratology study with Simazine technical was previously reviewed and classified as Core-Supplementary Data by Q. Q. Bui and L. D. Chitlik (memo, L. D. Chitlik, 7/3/85). It was indicated that this study may potentially be upgraded pending submission of an adequate explanation and/or clarification of several issues discussed below and a quality assurance statement.

The registrant, in response to EPA's review of this study, has submitted additional information and comments as an addendum in an effort to satisfactorily address the issues raised in the review.

1. The reviewers questioned the reported "statistically significant decrease in the number of viable fetuses in the intermediate and high dose groups" because the reported mean numbers of live fetuses for the control, low, mid and high dose groups were respectively 7.9, 8.4, 6.8 and 7.8. In this

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report, the study director agrees that the mean of 7.8 in the high dose group cannot be significantly different from the mean of 7.9 in the control group. He further explained that statistically significant differences between the control group and the mid and high dose groups were demonstrated only in a nonparametric analysis of covariance with the number of implantation sites as a covariant. Our review is now in agreement with the study director that there were no significant differences between control and treated groups in the mean number of live fetuses per litter.

2. Our review concluded that the investigator's calculation of pre- and post-implantation loss rates were incorrect. The reviewers determined that, instead of calculating loss percentages per dam and then deriving mean group loss percentages, investigators should have calculated group percentages of pre- and post-implantation losses using the total numbers of corpora lutea, implantations and viable fetuses per dose group. The study director disagreed and stated, "In almost all other calculations in studies of this nature, the litter is taken as the appropriate experimental unit. If one fails to use the litter as the experimental unit, they are assuming inaccurately that there do not exist any within litter correlations. Because we have appropriately used the litter as the experimental unit and because our mathematical calculations are correct (see attached tables), resubmitting re-calculations or re-analyses of this data would be inappropriate". He cited the following reference to support his rationale.

> D. W. Gaylor, "Methods and Concepts of Biometirics Applied to Teratology" in Handbook of Teratology, J. G. Wilson and F. C. Fraser, eds. (1978). Volume 4:Research Procedures and Data Analysis. Page 432.

Ciba-Geigy toxicologists responded in agreement with the study director that calculation of pre- and post-implantation loss rates for each entire dose group "assumes incorrectly that there can be no influence of litter size upon fetal and embryonic viability, and that the fetus, instead of the litter, is the experimental unit". The converse of these assumptions "are generally considered cornerstones in reproductive physiology".

The explanations of why percentages of pre- and post-implantation loss were calculated for each litter and averaged for the total number of litters in each treatment group are acceptable and adequately clarify the issue.

3. Recalculation and statistical re-analyses are deemed unnecessary because problems no longer exist for issues raised in items 1 and 2 above.

4. A copy of a Quality Assurance Unit Statement which is page 214 of the report in the registrant's archives was attached to its response to EPA's review of the Simazine rabbit teratology study.

Conclusions:

Simazine technical (97% purity) was not teratogenic in rabbits at dose levels up to and including 200 mg/kg when administered by gavage on days 7 through 19 of gestation. A significant reduction in mean fetal weight and a significant increase in the incidence of fetuses with skeletal variations were observed at the 200 mg/kg dose level. Significant decreases in body weight gain, tremors and abortions were observed in dams in the mid and high dose groups.

Teratogenic NOEL $\geq$ 200 mg/kg bw
Fetotoxic NOEL = 75 mg/kg bw (reduced mean fetal weight and increased skeletal variations at 200 mg/kg/bw.
Maternal NOEL = 75 mg/kg bw (decreased body weight gain, tremors and abortions at 75 and 200 mg/kg/bw).

Classification: Core - Guidelines

TS-769:ROBINSON:sll:X73710:5/8/86 Card Robinson

83-3

007240

Reviewed By: Quang Q. Bui, Ph.D.
Section I, Toxicology Branch - HFAS (H7509C)
Secondary Reviewer: Lawrence D. Chitlik, Senior Toxicologist
Science Analysis and Coordination Branch (H7508C)

5/12/89

DATA EVALUATION REPORT

Study Type: Teratology Study with Simazine in Rabbits

Accession No.: 252938

Comments By: Henry Spencer, Ph.D. 5/30/89
Section II
Toxicology Branch I - IRS (H7509C)

and

Marion P. Copley, D.V.M., Section Head 5-5-89
Review Section II
Toxicology Branch I - IRS (H7509C)

Conclusion:

This report No. 004535, as classified Supplementary represents a reasonable evaluation of the study.

Reviewed by Quang Q. Bui, Ph.D.
Secondary Reviewer: L. D. Chitlik, D.A.B.T.
Date: 7/3/85

007240

See Memorandum: Simazine, Teratology in Rabbits
study review -Document No. 004535

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

Data Evaluation Report

Chemical : Simazine Technical

Toxicity Chemical No. 740

Purity: Simazine Technical, 97% purity

Study Type: Teratology in rabbits

MRID No. —

Acc. No. 252938

Sponsor: Ciba-Geigy Corp.

Testing Facility: Ciba-Geigy Labs

Title of Report: A teratology study of Simazine technical in New Zealand white rabbits

Authors: Alan T. Arthus et al.,

Study No. 62-83

Report Issued: March 29, 1984

Conclusion:
The previous reviewers evauation (copy attached) accurately reflects the results of the study. The study is classified as core-supplementary.

"Under the conditions of this study, evidence of maternal toxicity was demonstrated at 75 and 200 mg/kg/day. Compound-related clinical manifestations (increased tremors and abortions) and significant decreases in food consumption and body wweight gain were noted at these two dosage levels during the dosing period. (7-19 d of gestation). The maternal NOEL was determined to be 5 mg/kg/day, (LDT). Although all fetal finding (fetal weight, skeletal variations and ossification centers) in the 5 mg/kg/day group were biologically similar to controls, A NOEL for developmental toxicity cannot be established at the present time pending the submission and/or clarification relative to several issues discussed below."

004535

STUDY REVIEW

Chemical: Simazine
Test Material: Simazine Technical, 97% purity
Study action/type: Teratology in rabbits

STUDY IDENTIFICATION:

A teratology study of Simazine technical in New Zealand white rabbits

Testing Facility: Ciba Geigy
Final Report No.: 62-83
Final Report date: 3/29/84
Study Directors: Alan T. Arthus et al.,
Accession No.: 252938

Study reviewed by:

Quang Q. Bui, Ph.D.
Section V, Toxicology Branch
Hazard Evaluation Division (TS-769C)

Reviewed and Approved by:

Laurence D. Chitlik, D.A.B.T.
Section Head, Section V
Toxicology Branch/HED (TS-769C)

CONCLUSIONS

Under the conditions of this study, evidence of maternal toxicity was demonstrated at 75 and 200 mg/kg/day. Compound-related clinical manifestations (increased tremors and abortions) and statistically significant decreases in food consumption and body weight gain were noted at these two dosage levels during the dosing period (days 7-19 of gestation). The maternal NOEL was determined to be 5 mg/kg/day (lowest dose tested).

A significant decrease in fetal weights and an increase in skeletal variations were observed at the highest dose tested (200 mg/kg/day). The mean numbers of resorptions were slightly increased at both the 75 and 200 mg/kg dosage levels as compared to controls. Although all fetal findings (fetal weight, skeletal variations and ossification centers) in the 5 mg/kg/day group were biologically similar to controls, a NOEL for developmental toxicity cannot be established at the present time pending the submission and/or clarification relative to several issues discussed below.

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1. Investigators' conclusion relative to mean number of live fetuses per litter:

The authors concluded that "fetal toxicity was evident in the intermediate and high dose groups as indicated by decreased numbers of viable fetuses" (page 6 of the final report) and "there was a statistically significant decrease in the number of viable fetuses in the intermediate and high dose groups" (page 13 of the final report). However, their statement was not substantiated by the submitted data since the mean number of live fetuses for the control, low, mid, and high dose groups was respectively 7.9, 8.4, 6.8, and 7.8 (Table 5, page 23 of the final report). A mean of 7.8 (high dose) could not be significantly different from 7.9 (control). In the absence of a dose-response relationship, the decrease noted in the mid- and high dose group was considered as of questionable importance in this review.

2. Calculation of pre- and post-implantation loss:

In this study, the group percentages of pre- and post-implantation loss were calculated from each dam, rounded-off, summed, and then divided by the number of dams in each group. The mean percentages of pre- and post-implantation loss were then reported in Table 5 (page 23) of the final report.

These calculations were thus not mathematically accurate due to "rounding errors". The percentages of pre- and post-implantation loss for each group should be calculated as follows:

$$\text{Pre-implantation loss} = \frac{\text{Total \# corpora lutea} - \text{Total \# implantations}}{\text{Total \# corpora lutea}} \times 100$$

$$\text{Post-implantation loss} = \frac{\text{Total \# implantations} - \text{Total \# viable fetuses}}{\text{Total \# implantations}} \times 100$$

When these indices were re-calculated using the above equations, mathematical differences were noted as indicated in the "Discussion" section of this review.

3. Statistical analysis of the results:

In light of the questionable statistical difference mentioned by the investigators in their conclusion (issue #1) and the newly obtained values for pre- and post-implantation loss after re-calculation using the appropriate equations (issue #2), re-calculation and re-application of statistical tests for all data apparently are necessary.

4. Quality Assurance Statement

A quality assurance statement was not appended with this report.

It is recommended that this study be classified as Core Supplementary Data. However, this study may potentially be upgraded pending the submission of:

(a) adequate explanation and/or clarification of the items listed above
(b) a quality assurance statement

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PROCEDURES

Test Material:	Simazine Technical, 97% a.i. Lot #821846, white powder
Animals:	New Zealand white rabbits (H.A.R.E.)
Dose levels:	0, 5, 75, and 200 mg/kg from days 7-19 of gestation
Vehicle:	3% corn stach containing 0.5% Tween 80
Route of administration:	gavage, 10 ml/kg/day

A copy of the procedures that were followed is appended. The study was basically conducted according to the 1982 FIFRA Guidelines and was of acceptable design. However, the following comments are noted:

Artifically inseminated animals were used but the method was not described in the study report. No data were available relative to the HCG injection and to the buck characteristics (number, health status, age, body weight at mating, semen collection, and semen analysis).

RESULTS

MATERNAL TOXICITY

1. Clinical Observations

Significant increases in the incidence of "little, none, and/or soft" stool were observed in the treated groups. These incidences were 0%, 50%, 100% and 100% for the 0, 5, 75, and 200 mg/kg groups, respectively. Compound-related increases in tremors were also noted being 0, 0, 21, and 100% for the 0, 5, 75, and 200 mg/kg groups, respectively.

2. Maternal death and abortion

Three animals died during this investigation. One each in the 5, 75, and 200 mg/kg groups. The authors indicated that the death observed in the 5 mg/kg group was accidental. Gross pathologic changes were not found in any animals at necropsy.

Four animals aborted, one dam from the 75 mg/kg group and three dams from the 200 mg/kg group.

3. Body Weights

Statistically significant decreases in maternal weight gains were found during the dosing period (days 7-19) in the 75 and 200 mg/kg groups. The corrected weight gain (body weight gain minus gravid uterine weight) of these two highest dosage levels were also significantly different from controls. Further, as shown in the following table, compensatory increases with statistical differences were observed in these two highest dose groups as compared to controls after cessation of the test material administration (days 17-29 of gestation). Therefore, the decrease in maternal weight gain observed in the 75 and 200 mg/kg groups during the dosing period (days 7-19) was compound-related.

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Maternal Body Weight Gain (grams)

	Control	5 mg/kg	75 mg/kg	200 mg/kg
Days 0-7	143	138	142	155
Days 7-19°	230	244	-243*	-456*
Days 19-29°	163	190	390*	436*
Days 0-29°	536	572	142*	135*
Corrected weight gain °°	3	3	-167*	-264*

(°): Calculated by the reviewers
(°°): Body weight gain minus gravid uterine weight
(*): Significantly different from controls, $P < 0.05$

4. Food Consumption

Significant decreases in food consumption were noted in the 75 and 200 mg/kg groups as compared to controls and persisted throughout the dosing period (days 7-19). The food consumption of the 5 mg/kg group was comparable to that of the control group.

5. Reproductive status at laparotomy

The reproductive status data are summarized in the next table.

Reproductive Status At Necropsy

	Control	5 mg/kg	75 mg/kg	200 mg/kg
# dams inseminated	19	19	19	19
# dams pregnant	18	18	18	16
Pregnancy index (%)	95	95	95	84
# dams aborted	0	0	1	3
# dams pregnant and dead	0	1	1	1
# litters examined	18	17	16	12
$\bar{X}$ corpora lutea	12.9	12.5	12.3	12.6
$\bar{X}$ implantations	8.8	9.5	9.4	10.3
$\bar{X}$ pre-implantation loss	4.2	2.9	2.9	2.3
Pre-implantation loss(%)°	28.8	23.6	22.7	17.0
$\bar{X}$ resorptions	0.8	1.1	2.7	2.5
$\bar{X}$ dead fetuses	0.0	0.5	0.0	0.0
$\bar{X}$ post-implantation loss	0.8	1.6	2.7	2.5
Post-implantation loss (%)°	11.5	18.1	25.1	22.6
$\bar{X}$ live fetuses	7.9	8.4	6.8	7.8
Sex ratio (% M)	54.5	52.6	48.1	54.3

(°) Calculated by the investigators - See "Discussion" section

The pregnancy index in all tested groups was within the acceptable range for artificially inseminated rabbits. No significant variations with respect to the mean numbers of corpora lutea, implantations, and live fetuses per dam were found among the control and treated groups.

Increases in the mean number of resorptions per litter and, hence, post-implantation were noted in all treated groups. Although, a clear dose-response was not demonstrated from the reported data, it is still apparent that the increased resorptions were compound-related at least for the 75 and 200 mg/kg groups. Implantation loss on a per litter basis for the 0, 5, 75, and 200 mg/kg groups were respectively 0.8, 1.6, 2.7, and 2.5. Although increased resorptions were noted in treated groups, no significant decrease in the mean number of live fetuses was observed in the treated groups since all treated groups had higher implantation rates than control.

The investigators' statement that "a statistically significant decrease in the number of viable fetuses in the intermediate and high dose groups" (page 13 of the final report) was thus not substantiated by the study results which were 7.9, 8.4, 6.8, and 7.8 for the 0, 5, 75, and 200 mg/kg groups, respectively. Therefore, a mean of 7.8 (high dose) could not be significantly different from 7.9 (control). In the absence of a dose-response relationship, the apparent decrease in the mean number of viable fetuses in the mid- and high dose groups was considered as of questionable importance. No alterations in fetal sex-ratio were found.

DEVELOPMENTAL TOXICITY

1. Fetal Weight

As illustrated in the next table, a significant decrease in fetal weight was observed only in the 200 mg/kg group. This decrease did not result from a compensatory effect with respect to increase in litter size and, hence, was considered as a compound-related effect.

Fetal Weight (grams)

	Control	5 mg/kg	75 mg/kg	200 mg/kg
Male	45.4	45.2	46.3	41.2
Female	45.5	44.4	44.3	39.4*

(*): Significantly different from controls, $P < 0.05$

Crown-rump length was not measured in this investigation.

2. Malformations

Two fetuses were found with external malformations. One fetus from the 75 mg/kg group was described with acrania and one 200 mg/kg fetus had multiple malformations (atretostomia, excencephaly, and protruding tongue). That same fetus of the 200 mg/kg group was also described with malposition of the umbilicus, micropthalmia, and malformed mandible.

In summary, no biological or statistical increases in the incidences of either litters or fetuses with malformations were found up to and including a dosage level of 200 mg/kg/day in rabbits.

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3. Variations

Skeletal variation findings of interest are summarized in the next table:

Skeletal Variations

	Control	5 mg/kg	75 mg/kg	200 mg/kg
# fetuses examined	143	135	108	94
# litters examined	18	16	16	12
13th full ribs				
fetuses (%)°	27(19)	30(22)	38(35)	44(47)*
litters (%)	13(72)	12(75)	13(81)	12(100)
Ribs, free floating				
fetuses (%)	0(0)	0(0)	1(0.9)	3(3.2)
litters (%)	0(0)	0(0)	1(6.3)	2(16.7)*
Sternebrae non-ossified				
fetuses (%)	2(1.4)	7(5.2)	5(4.6)	8(8.5)
litters (%)	2(11)	4(25)	5(31)	4(33)
Sternebrae misaligned				
fetuses (%)	24(17)	37(27)	23(21)	22(23)
litters (%)	9(50)	9(56)	9(56)	8(67)
Patella non-ossified				
fetuses (%)	2(1.4)	0(0)	8(7.4)	25(26.6)
litters (%)	2(11)	0(0)	3(19)	7(58)*
Talus/Calcaneus not ossified				
fetuses (%)	1(0.7)	0(0)	0(0)	6(6.4)
litters (%)	1(5.6)	0(0)	0(0)	2(16.7)
Total fetuses affected	71(50)	86(64)	70(65)	83(88)*
Total litters affected	18(100)	16(100)	16(100)	12(100)

(*): Significantly different from controls, $P < 0.05$
(°): Percentages calculated by these reviewers

Compound-related increases in the incidences of fetuses with 13th full ribs, sternebrae non-ossified, ribs free-floating, and patella non-ossified were found at the 75 and 200 mg/kg dose levels. Also, a significant increase in the incidence of fetuses with any variations was noted at the 200 mg/kg dosage level.

DISCUSSION

Under the conditions of this study, maternal toxicity was characterized by significant decreases in food consumption and body weight gain during the dosing period (days 7-19) in dams treated with 75 and 200 mg/kg/day and by the presence of toxic manifestations (decreased motor activity and tremors) at the 200 mg/kg dosage level. It is suggested that the maternal NOEL be established at 5 mg/kg (lowest dose tested).

Dosage levels of 75 and 200 mg/kg were associated with a slight increase in the numbers of resorptions per dam as compared to controls. Also, a significant reduction in fetal weight was also observed at the 200 mg/kg/day dose level.

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Teratogenesis was not evident at any of the dosage levels tested (highest dose tested = 200 mg/kg/day).

A developmental toxicity NOEL could not be established at the present time due to the following:

1. The study authors' statements that "fetal toxicity was evident in the intermediate and high dose as indicated by decreased numbers of viable fetuses" (page 6) and "statistically significant decrease in the number of viable fetuses in the intermediate and high dose groups" (page 13) were not supported by the submitted data. The mean number of viable fetuses for the 0, 5, 75, and 200 mg/kg groups were respectively 7.9, 8.4, 6.8, and 7.8. Significant differences, as stated by the investigators, are not apparent.

2. Our review disagrees with the investigators' calculations of the group percentages of pre- and post-implantation loss.

In this study, for example the pre-implantation was calculated as follows (page 13) :
% pre-implantation loss=

$$\frac{\text{No. corpora lutea - No. of implantation sites x 100 (per dam)}}{\text{No corpora lutea}}$$

Therefore, the percentage of pre-implantation loss for each dam was calculated, rounded off, summed, and divided by the number of dams. The mean percentages of pre- or post-implantaion loss were then presented in Table 5.

This calculation is not mathematically accurate due to "rounding errors". The group percentages for pre- and post-implantation should be calculated as:

Pre-implantation loss:

$$\frac{\text{Total \# corpora lutea - Total \# implantations x 100}}{\text{Total \# corpora lutea}}$$

Post-implantation loss:

$$\frac{\text{Total \# implantations - Total \# viable fetuses x 100}}{\text{Total \# implantations}}$$

Example:

Post-implantion loss (%)	Final Report °	Recalculated
Control	11.5	9.5
5 mg/kg	18.1	16.6
75 mg/kg	25.1	28.4
200 mg/kg	22.6	24.2

(°): Data extracted from Table 5

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3. In light of the above issues, the registrant is requested to re-calculate the indices of pre- and post-implantation loss. All data, including those of mean viable fetuses per litter, should be statistically re-analyzed.

4. A "Quality Assurance" statement should be appended with the final report.

In conclusion, the above listed issues and discrepancies must be adequately resolved by the registrant before upgrading of this report can be considered.

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Simazine RIN: 0569-93

Page_____ is not included in this copy.

Pages 203 through 206 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.

✓ 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

Reviewed By: Henry W. Spencer, Ph.D. 4/24/89 007240
Review Section II, Toxicology Branch I - IRS (H7509C)
Secondary Reviewer: Marion P. Copley, D.V.M. 5/2/89
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

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 81 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.

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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%	88%	89%	87%	84%
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.

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R:57920:Spencer:C.Disk:KENCO:04/24/89:CL:VO:EK:CL

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~~Attachment 25~~

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

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

06/--/87

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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007240

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
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Date: 7-29-88

6. APPROVED BY:

I. Cecil Felkner, Ph.D.
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Dynamac Corporation

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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

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 approoriate 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.

C. REPORTED RESULTS:

1. 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 I. 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 RIN: 0569-93

Page_____ is not included in this copy.

Pages 220 through 226 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.

__✓__ 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.

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CONFIDENTIAL
NATIONAL SECURITY INFORMATION (EO 12065)

06/30/89

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

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007240

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.
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Dynamac Corporation

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

6. APPROVED BY:

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Date: 8-3-88

Henry Spencer, Ph.D.
EPA Reviewer

Signature: [illegible]
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., [illegible]z, 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.

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

b. Hepitocyte 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 $p \leq 0.01$.

B. 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 RIN: 0569-93

Page_____ is not included in this copy.

Pages 235 through 240 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.

✓ FIFRA registration data.

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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.

Attachment 6

087402

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

06/30/89

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

007240

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

007240

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:

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Date: 8-3-88

I. Cecil Felkner, Ph.D.
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Date: 8-3-88

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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 B, Analytical Data, CBI pp. 18-21.)

[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).

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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 RIN: 0569-93

Page_____ is not included in this copy.

Pages 249 through 256 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.

✓ 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.

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Reviewed By: Brian Dementi, Ph.D. 12/16/86 (P...)
Section 1, Toxicology Branch (TS-769C)
Secondary Reviewer: R. Bruce Jaeger 12/17/86
Section 1, Toxicology Branch (TS-769C)

DATA EVALUATION REPORT

Study Type: Metabolism (Disposition in the Rat)

Accession Number: 262646
MRID 00143266

Test Material: Simazine

Synonym: 2-chloro-4,6-diethylamino-s-triazine

Caswell Number: 740

Project Number: 1852

Study Number: ABR-86032

Sponsor: Ciba-Geigy Corporation, Greensboro, NC.

Testing Facility: Stanford Research Institute (SRI), Menlo Park, CA and Ciba-Geigy Laboratories, Greensboro, NC.

Title of Report: Disposition of Simazine in the Rat.

Author(s): G.R. Orr and B.J. Simoneaux

Date Issued: April 30, 1986

Conclusions:

At the low dose of administration (0.5 mg/kg) of ^{14}C-radiolabeled simazine, the principal route of excretion was via the urine, however, at the higher dose (200 mg/kg) the principal route of excretion was via the feces. Significant radioactive residues remained in the tissues of the rat for extended periods of time. Results indicate that 94 to 99 percent of the elimination of radioactive material occurred within 48 to 72 hours with a half-life of 9 to 15 hours. Elimination of the remaining radioactivity exhibited 21- to 32-hour half-life values. Heart, lung, spleen, kidney, and liver appear to be principal sites of retention of radioactivity. However, erythrocytes concentrated radioactivity to higher levels than did other tissues, perhaps due to high affinity of the triazine ring for cysteine residues of hemoglobin, a phenomenon apparently unique to rodent species.*

In addition to the single studies at the two doses indicate a third study conducted at the same lower dose, but preconditioned with cold simazine for 14 days in general affirmed that saturation of binding sites occurs in most tissues examined. An exception was the erythrocyte, where following preconditioning with cold simazine, radiolabeled simazine was more effectively retained in comparison to other tissues. This suggests a greater potential for erythrocytes to accumulate this compound.

Classification: Core - Minimum

Special Review Criteria

A. Materials

1. Test Compound: Radiolabeled simazine (^{14}C-triazine ring), Description: 0.83 Ci/mg (high dose expt.) and 15.6 Ci/mg (low dose expt.), Purity = ≥ 98% radioactive purity

2. Test Animals: Species: Rat, Strain: Charles River CD, Weight: 160-225 g, Source: Charles-River Breeding Laboratory, Wilmington, MD

B. Study Design:

1. Animal Assignment - Animals were assigned 5 (M,F) each to the following test groups:

Test Group		Dose
a.	Control	Dosing vehicle
b.	Low	0.5 mg/kg (15.6 Ci/mg), single dose
c.	High	200 mg/kg (0.83 Ci/mg), single dose
d.	Preconditioned	14 days with 0.5 mg/kg unlabeled simazine followed by 0.5 mg/kg (15.6 Ci/mg), single dose

Compound administered orally via stomach tube, vehicle was polyethylene glycol (Carbowax 200)

2. Diet Preparation - Test compound was not administered via diet.

3. Animals received food, "Standard Laboratory Diet," and water ad libitum.

4. Statistics - Statistical methods of analysis employed not discussed. At certain points in the discussion of the data, the authors refer to significant differences between various groups, but results as tabulated do not identify which differences are statistically meaningful.

5. Quality assurance was addressed by the manager of Regulatory Affairs and Quality Assurance. It was certified that "any deviations from the approved protocol and standard operating procedures were made with proper authorization and documentation."

C. Methods and Results:

Urine and feces were collected at specified time points during the 7-day dosing period for radioassay. Also, at the time of sacrifice, cages were washed with acetone/water mixture for radioassay. At the termination of the dosing period, blood was obtained from each animal and was fractionated into plasma and red blood cells for radioassay. All animals were then sacrificed and portions of selected tisues removed in the indicated order for purposes of radioassay: heart, lung, spleen, kidneys, liver, fat, gonads, uterus, muscle, brain, bone, and carcass.

Radioactivity Analysis: Tissue and feces were homogenized by grinding at liquid nitrogen temperature. Weighed portions of these homogenates were combusted and counted. Urine and cage wash samples were counted directly. Counting was perfcrmed using a Beckman Model 3801 Liquid Scintillation Counter and efficiency was determined by external standardization.

Results: Total recovery of radiolabel ranged from 78.3 to 91.5 percent. Excretion via the urine was the preferential route of elimination for the low and preconditioned low dose studies (50.5 to 66.0%), females exhibiting the higher levels in this range. However, in the high dose experiment, excretion was primarily via the fecal route (54.9 to 63.2%) with females displaying the higher values. These higher values for females are probably not statistically significant findings.

Just why the principal route of elimination shifts from the urine at the low dose to the fecal route at the high dose cannot be satisfactorily explained given the limited information available. The shift could be speculated as due to saturation of metabolic capability via the urinary route, or perhaps as due to limited solubility or poor absorption of the compound by the G.I. tract.

Radioactive residues remained in the tissues of animals from all three test groups. These values ranged 2 percent in the high dose group, 8 percent in the preconditioned dose groups, and 12 percent in the low dose group (re: Table 1). Lower retention levels in the high and preconditioned-low dose groups are considered to be due to relative saturation of simazine binding sites. The study authors indicate (p. 6) that highly perfused, metabolically active tissues such as liver, kidney, lung, and heart in addition to erythrocytes show significant residues in all three test groups. The spleen should also be included in this observation (re: Table 2-3, Fig. 1-2). It should be noted, however, that Tables 2 and 3 referred to by the authors as supporting this claim, do not indicate which points are significantly different.

An interesting finding was that erythrocytes have greater potential to accumulate or concentrate simazine than the other tissues examined. The study authors cite a publication* which is claimed to indicate this as due to "Covalent binding of the triazine ring to an exposed cysteine residue in the chain of rodent hemoglobin. This sulfhydryl moiety is apparently unavailable for binding in the hemoglobin of other mammalian species." A copy of this cited publication has been requested for Toxicology Branch inspection.

Reference

* Hamboeck, H.; Fischer, R.W.; Di Iorta, E.E.; Winterhalter, K.W. (1981) Molecular Pharmacology, 20, 579-584.

Attachment "B"

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NOTE TO FILE Simazine, Caswell #740

Comment upon simazine metabolism study entitled "Disposition of Simazine in the Rat" Accession No. 262646; Study No. ABR-86032

MRID 00147266

A prinicipal finding in this study was that among the various tissues assayed for the presence of radiolabel following the administration of ^{14}C- simazine, levels were highest in red blood cells (pp. 6, 19-22). Ciba-Geigy attributes this to a unique binding of the triazine ring to exposed cysteine residues located on the β chain of hemoglobin. Furthermore, the study authors claim this binding is peculiar to rodent hemoglobin as opposed to that of other species. In support of this contention the authors cite a published work by Hamboeck, et al (1981). This particular publication did not accompany the metabolism study as submitted by the petitioner. We have now obtained and reviewed a copy of this article.

The published work by Hamboeck, et al indicates that a unique binding of triazines to rodent hemoglobin as opposed to human or other mammalian hemoglobin appears contingent upon the presence of an alkylthio- or other thio- substituent at position 6 of the triazine ring, which is metabolically oxidizable to the sulfoxide. ~~It is the sulfoxide which binds~~ the β chain of hemoglobin. Simazine does not have a thio- substituent at any locus on the triazine ring, and furthermore is chlorinated at position 6. This would not appear to enable simazine to bind the β chain of hemoglobin in the manner described by Hamboeck, et al. In addition, the authors state that s-triazines, per se, do not appreciably bind to the contents of erythrocytes (p. 582).

Thus it would appear that high levels of simazine found in rat red blood cells is not adequately explained by the selective binding phenomenon noted for thio- substituted triazines, and would make it doubtful that the simazine binding observed with rat hemoglobin would not occur in human or other non-rodent red cells.

Brian Dementi, Ph.D.
Review Section 1

Brian Dementi 1-15-87

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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 evauation (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 asscciated with dermal exposure.

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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/cm^2 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

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Data Evaluation Report

Compound tested Simazine

Citation
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.

8/54/88

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 treratment." The high dose was admiinistered 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, stoma-hesive 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	actual 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	potential absorbable
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.

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