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

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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.
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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

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.

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 inidividually 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 grcups 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 initiatin 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 on weekends and holidays. Pre-dose body weights and weekly body weights and food consumption were recorded.

Ten fasted 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.

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 caculated 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 239.2 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% & 32.1%) 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 levls 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.

Sex	Number of renal calculi at doses (ppm) of 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 attendng 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.

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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 healthy 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.

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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 9 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

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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.

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3. Metabolism of Simazine and its Metabolites in Female Rats by B. Simoneaux and A. Sy, Geigy Agricultural Chemicals Division, Ciba-Geigy Corp., Rep. No. GAAC-71030, May 31, 1971; Acc. No. 257694.

Test Materials:

a. ^{14}C ring-labeled simazine (G-27692), 99.8% purity

b. aqueous and insoluble fish metabolites from a previous experiment (Simoneaux & Sy, 1971).

Test Animals: Female white rats (Charles River) on a diet of hard sticks of Purina rat chow and housed in Roth Metabolism cages.

Experimental Design:

All rats were dosed once by stomach tube as follows:

a. Two rats (A & B) received 1.5 mg/kg of ^{14}C ring-labeled simazine (G-27692);

b. Rat C received 0.101 mg/kg of aqueous fish metabolites which were extracted from 28-day bluegill muscle;

c. Rat D received 0.028 mg/kg of insoluble fish metabolites which were a mixture of solids obtained by multiple extraction of 7, 14 and 28-day depletion bluegill fish; and,

d. Rat E received 0.008 mg/kg of a mixture of aqueous and insoluble fish metabolites obtained from a 21-day treatment catfish.

Rats C, D and E were fed fish biosynthesized ^{14}C-metabolites to verify the belief that this material is not biologically available to these rats.

Rats dosed with ^{14}C-simazine were fed sticks fortified with 15 ppm cold G-27692. The simazine dosed rats were fed a nonfortified feed stick on the day of dosing. Urine, feces and respiratory CO_2 were collected separately from the Roth Metabolism cages. Rats C, D and E were sacrificed 72 hours after dosing. The simazine dosed rats (A & B) were sacrificed after 96 hours. Blood and several tissue samples were taken and stored at -20 °C until radioassayed. At termination, all cages were washed with water and the wash diluted to a known volume.

Urine and cage washes were radioassayed by direct counting in a liquid scintillation counter. Blood, tissues and feces were radioassayed by combustion. Respiratory $^{14}CO_2$ was collected in phenethylamine after acid release and assayed. Metabolic characterization of the initial 24-hour urine from ^{14}C-simazine dosed rats (A & B) was conducted by one- and two-dimensional chromatography. Total triazine residues in 24-hour urine from ^{14}C-simazine dosed rats (A & B) were converted to cyanuric acid and quantified by two-dimensional chromatography (TLE/TLC).

Results:

The total average recovery of radioactivity for rats A & B dosed with ^{14}C-simazine was 99%. The total radioactivity recovered from the radioactive metabolites of simazine biosynthesized by fish in rats C, D and E ranged from 78% to 125%. Rats dosed with ^{14}C-simazine excreted 41% of the radioactivity in the feces and 49% in the urine. Rats dosed with radioactive fish metabolites excreted 48 to 93% of the radioactivity in feces and 17 to 31% in urine. The amount of expiratory $^{14}CO_2$ was less than 0.5% of the radioactive dose in all rats.

Radioactivity of tissues and blood from rats dosed with simazine was 5.2 and 2.6%, respectively. Tissue radioactivity from rat C dosed with aqueous fish metabolites was 12.9% and less than 0.05% in blood.

Examination of the 24-hour urine sample of one rat dosed with simazine revealed a pattern of metabolites as follows: < 1.0% chlorotriazines, 27% hydroxytriazines, 20% hydrolyzable conjugates, and 57% unknown soluble metabolites.

Conclusion:

It appears, from this abbreviated study, that the overwhelming majority (90%) of ^{14}C-simazine and its metabolites are excreted fairly equally in the feces and urine of dosed rats. Only small amounts were detectable in blood and a few selected tissues 96 hours postdosing. However, this study does not satisfy requirements as set forth in Section 85-1 Metabolism Study for the following reasons:

a. The number of animals/sex, animal groups and dose levels were too few. At least 10 rats (5/sex) should be used at at least 2 dose levels. The following four groups of animals should be studied:

   Group A. These animals should each receive a single intravenous dose of the labeled substance at the low dose

Group B. These animals should each receive a single oral dose (by capsule or intubation) of the labeled test substance at the low dose.

Group C. These animals should each receive a series of single daily oral doses of the nonlabeled test substance (by capsule or intubation) over a period of at least 14 days, followed by a single oral dose (by capsule or intubation) of the labeled test substance. Each dose should be the low dose level.

Group D. These animals should each receive a single oral dose (by capsule or intubation) of the labeled test substance at the high dose level.

b. Measurement intervals and observation period were inadequate. Animals should be kept in individual metabolism cages for 7 days after radioactive dose or until 90+ percent of the administered dose is excreted (whichever occurs first). Quantities of the label in urine, feces, and expired air should be measured at appropriate intervals (e.g., 4, 8, 12 and 24 hours, 1.5, 2, 3, 4, 5, 6 and 7 days) throughout the study for all animals.

Classification: Core-Supplementary Data.

4. Simazine Technical Acute Inhalation Toxicity Study in Rats by C. Breckenridge, J. Scott, M. Mehesy and J. Green; Ciba-Geigy Pharmaceuticals Research, Safety Evaluation Facility, Summit, NJ, Tox/Path Report No. 216-84, October 18, 1984; Acc. No. 255268

Test Material: Simazine Technical, Batch No. FL-841668, white powder, purity of 97%.

Test Animals: Male and female Sprague-Dawley [Crl: COBS® CD® (SD) BR] rats. Males were 8-9 weeks old weighing 309-313 g; females were 9-12 weeks old weighing 226-228 g. Rats were caged individually under standard controlled laboratory conditions with feed and water ad libitum.

Experimental Design:

Five rats/sex were assigned each to a test group and a control group. Each group (5M & 5F) was housed in one of two whole body exposure chambers. The test group was exposed to a chamber concentration in excess of 2 mg Simazine Technical/liter of room air for a period of 4

consecutive hours. The control group was exposed only to room air under the same conditions. The air flow rate was set at 60 liters/ minute and monitored every 30 minutes.

Results:

Hourly gravimetric chamber concentrations of simazine ranged from 1.9 to 2.3 mg/l with a 4-hr average of 2.1 mg/l. Median particle diameter (hourly) ranged from 4.8 to 5.9 um with 72% of particles being < 9 um in diameter. The 4-hr nominal chamber concentration was 4.4 mg/l of air.

Simazine was present on the fur of test animals when removed from the chamber. Nasal secretions, salivation, frequent urination, soft feces and general unkempt appearance were among signs observed in treated rats during the first 4 days postexposure. Control animals appeared normal from initiation to termination.

There were no appreciable differences in body weights, lung weights and gross pathology between treated and control rats.

Conclusion:

LC_{50} > 2.1 mg/liter

Classification: Core-Minimum Data; Category III

NOTE: The single dose of 2.1 mg/liter is much less than the "Test Limit" of 5.0 mg/liter as prescribed in Pesticide Assessment Guidelines, November 1982. However, Toxicity Category III covers LC_{50} of 2-20 mg/liter. Therefore, even the test limit of 5.0 mg/liter would be in this toxicity category.