

CHEM Chlorosulfuron

002653

BRANCH TB DISC

TOPIC 2-Year Feeding - Rat,
3-Generation Reproduction - Ra

FORMULATION Technical

FICHE/MASTER ID

CONTENT CAT

Long-Term Feeding Study With 2-Chloro-N-[(4-Methoxy-6-Methyl-1,3,5-Triazin-2-Yl)Aminocarbonyl]Benzenesulfonamide (INW-4189) In Rats, Haskell Laboratory Report No. 557-81, Wood, C. K.

SUBST. CLASS =

OTHER SUBJECT DESCRIPTORS

DIRECT RVW TIME = 10 hours

START-DATE

END DATE

REVIEWED BY: J. C. Summers

TITLE: Research Associate

ORG: E. I. du Pont de Nemours & Co., Inc., Biochemicals Dept.

LOC/TEL: Wilmington, Delaware / (302) 772-2367

SIGNATURE: *J. C. Summers*

DATE: November 10, 1961

APPROVED BY:

TITLE:

ORG:

LOC/TEL:

SIGNATURE:

DATE:

Conclusion:

002653

2-Year Feeding - Rat

- A. Core Guideline
- B. A NOEL of 100 ppm was established based on body weight loss when technical chlorsulfuron was fed to rats for 2 years at dietary levels of 0, 100, 500, or 2500 ppm. No gross or histopathological abnormalities that could be attributed to chlorsulfuron were observed at any test level.
- C. This study conforms to EPA Proposed Guidelines in Section 163.83-1 Chronic Feeding Study (43 Federal Register 37375, 8/22/78).

Methods:

2-Year Feeding - Rat

Three hundred sixty-eight male and three hundred and seventy-one female CD® rats were received from Charles River Breeding Laboratories, Wilmington, Massachusetts. Following a twelve day pretest, 320 rats of each sex, selected on the basis of weight gain and freedom from signs of disease or injury, were divided by randomization into four groups of 80 males and 80 females and housed in pairs. Groups were fed ground Purina® Laboratory Chow diets containing 0, 100, 500, or 2500 ppm chlorsulfuron for two years. Diets were prepared fresh weekly and stored under refrigeration until used. Rats received test diet and water ad libitum.

All rats were examined at least once daily during the first 14 weeks of the study and at least twice daily after that for abnormal behavior and clinical signs of toxicity. Rats were weighed once a week during the first six months, once every two weeks during the next six months, and once every four weeks during the last 12 months. Diet consumed by rats was determined on a group basis at each weighing interval, and food efficiency and daily intake were calculated. Mortality was recorded.

Three, six, twelve, eighteen, and twenty-four months after initiation of the study, ten rats from each of the study groups were subjected to clinical chemistry, hematological and urine analytical examinations. Tail blood was evaluated for alkaline phosphatase, SGOT, SGPT activities, BUN, creatinine, and total plasma protein. Urine, from the same animals was collected in the same time intervals and examined for volume, pH, sugar, protein, bilirubin, urobilinogen, occult blood, color, appearance, and sediments. The blood from this same group of animals was examined for the following parameters: erythrocyte, leukocyte, and differential leukocyte counts, hematocrits, and hemoglobin concentrations. Reticulocyte counts were performed at the 18- and 24-month examination periods. Mean corpuscular hemoglobin, mean corpuscular volume, and mean corpuscular hemoglobin concentrations were calculated.

After fifty-two weeks, ten rats not subjected to the clinical tests were selected from each test group, sacrificed, and necropsied. Rats found dead or sacrificed in extremis during the study were sacrificed and necropsied. The brain, heart, spleen, thymus, stomach, pituitary, adrenals, lungs, liver kidneys, and testes were weighed and mean final body weights, organ weights, and organ to body weight ratios were calculated. The tissues noted

above and the following tissues were examined microscopically for all rats at all feeding levels for histopathologic changes: spinal cord, sciatic nerve, aorta, mesenteric vessels, sternbrae, and humoral bone marrow, sternbrae, lymph nodes, eye, skin with underlying mammary tissue, skeletal muscle, salivary and exorbital lacrimal glands, esophagus, duodenum, jejunum, ileum, cecum, colon, pancreas, thyroid and parathyroid glands, trachea, urinary bladder, prostate, epididymides, testes, mammary glands, ovaries, uterine horns, vagina and all masses.

Results:

2-Year Feeding - Rat

A mild to moderate reduction in mean body weights and weight gains occurred in male rats fed 2500 ppm and 500 ppm chlorsulfuron. The male rats at 100 ppm and the female rats at all dose levels were comparable to their respective control groups throughout the study. Diet consumption was comparable between control and test groups, but food efficiency was decreased in the 2500 ppm male group. Clinical signs, incidence of palpable tissue masses and mortality were comparable in test and control groups.

MALE BODY WEIGHTS

<u>Time (Weeks)</u>	<u>0</u>	<u>100</u>	<u>500</u>	<u>2500</u>
0	119.5	119.5	119.4	119.4
6	361.9	362.3	349.1*	344.1*
13	491.3	493.0	481.8	471.8*
26	580.3	578.9	572.7	554.2*
52	695.2	677.1	669.2*	637.8*
76	768.9	748.8	730.5*	702.9*
104	751.1	754.9	710.5	721.2

* Different from control at P \leq .05 level of significance

INCIDENCE AND MEDIAN TIME TO ONSET OF PALPABLE TISSUE MASSES

<u>Treatment Group</u>	<u># Masses</u>	<u># Animals Affected</u>	<u>Median Time to Onset (wks); Range</u>
<u>Male</u>			
Control	15	14	72; 42-100
100 ppm	12	11	72; 42-104
500 ppm	5	5	100; 88-100
2,500 ppm	16	14	88; 60-104
<u>Female</u>			
Control	35	29	84; 52-100
100 ppm	39	33	76; 44-100
500 ppm	36	28	88; 11-104
2,500 ppm	31	26	76; 42-104

SURVIVAL

002653

<u>Treatment Group</u>		<u>#Rats Alive at Study End (Week 104)</u>
<u>Male</u>	<u>Median Survival Time (wks)</u>	
Control	101.0	46
100 ppm	104.5	44
500 ppm	101.5	41
2500 ppm	105.0	47
<u>Female</u>		
Control	102.0	44
100 ppm	102.5	44
500 ppm	101.5	42
2500 ppm	107.0	46

During the first year of the study, male rats fed 500 and 2500 ppm of test compound exhibited dose-dependent decreased erythrocyte counts, increased hematocrits, mean corpuscular volumes and corpuscular hemoglobins, and slightly decreased mean corpuscular hemoglobin concentrations. This was suggestive of reticulocytosis. However, during the second year of the study, these abnormalities were not observed. In addition, no meaningful differences in reticulocyte counts were observed between control and test groups at 18- or 24-month examinations.

No gross or histopathological abnormalities were considered compound-related. (The pathologist concluded that the test material was not observed to be carcinogenic under conditions of the study.) A summary of incidence of microscopic observations is attached at the end of this evaluation.

In the absence of dose relatedness in the absolute and relative kidney weights of the male test groups, slight decreases observed were not considered to be compound-related. Male rats in the 2500 ppm group exhibited a higher incidence (13/69) of unilateral interstitial cell tumors than was observed in control group males (2/68). However, this was not considered compound-related since a compound-induced effect would be expected to affect the testes bilaterally. The incidence of bilateral interstitial cell tumors in male rats from the control group (7/68) was greater in male rats from the 2500 ppm group (3/69). Also the unilateral incidence was within the known spontaneous range for CD⁰ rats and there were no other changes such as interstitial cell hyperplasia suggestive of a compound-related tumorigenic effect in the testes. A NOEL of 100 ppm was established based on body weight loss.

Discussion:

2-Year Feeding - Rat

The methods and materials, scientific principles, validity of conclusions, and adequacy of data for conclusions were adequate for the

study. The reviewer agrees with the conclusions of the study. HLR 283-80 is a one-year interim report issued on the two-year rat study, but it was not reviewed since it was superceded by two-year report 557-81. The study was run under Medical Research Project No. 3067.

002653

Results:3-Generation Reproduction - Rat

Rats in the 2500 ppm group had slightly decreased fertility indices when compared to controls. Mean number of pups/litter, gestation, lactation, and viability indices, litter survival, mean weanling body weights and weight gains, diet consumption and food efficiency were not adversely influenced. Slight differences in mean weanling body weights in the 2500 ppm group were not consistently related to dietary concentrations and were not considered biologically significant. Clinical observations were not dose-related and were not considered to be compound-related. Mean organ weights and organ weight ratios of weanling rats from the test groups were comparable to those of the controls. No gross or histopathological abnormalities that could be related to dietary administration were observed in the F_{3b} weanlings. The no observable effect level was considered to be 500 ppm based on decreased fertility indices.

<u>Treatment Group (ppm):</u>	<u>0</u>	<u>100</u>	<u>500</u>	<u>2500</u>
<u>Litter</u>	<u>Fertility Index (%)</u>			
F _{1a}	95	90	95	95
F _{1b}	100	95	95	89
F _{2a}	95	90	85	84
F _{2b}	100	95	89	100
F _{3a}	95	100	90	79
F _{3b}	95	100	100	79

Discussion:3-Generation Reproduction - Rat

The methods and materials, scientific principles, validity of conclusions, and adequacy of data for conclusions were adequate for the study. Modifications in the guideline such as reproduction through 3 generations rather than the guideline's two, breeding twice within each generation to produce F_a and F_b litters, and doing histopathology on F_{3b} weanlings rather than on weanlings of each generation are variations which do not affect the validity of the study. This study was reviewed in connection with teratology study HLR-583-78.

Conclusion:3-Generation Reproduction - Rat

- A. Core guideline
- B. A NOEL of 500 ppm based on decreased fertility indices was established when technical chlorsulfuron was fed to rats in a 3-generation 6-litter reproduction study at dietary levels of 0, 100, 500 and 2500 ppm.
- C. This study generally conforms to EPA proposed guidelines in section 163.83-4 Reproduction study (43 Federal Register 37384, 8/22/78) with some modifications.

Methods:3-Generation Reproduction - Rat

Charles River CD® male and female rats were fed diets that contained 0, 100, 500 or 2500 ppm chlorsulfuron in a long term feeding study. After 103 days of feeding, 20 rats/sex/level were selected for the three-generation six-litter reproduction study and temporarily removed from the long-term study.

Male and female F₀ rats within each dietary group were mated for a 15-day period to produce F_{1a} litters. During the mating and reproduction phases, F₀ rats continued to receive their respective test group's diets. Approximately seven days after weaning the F_{1a} litters, the F₀ rats were mated a second time for a 15-day period to different rats to produce F_{1b} litters. At weaning, the F₀ rats were returned to their respective groups in the long-term feeding study. The number of pups in each F_{1b} litter was reduced to ten and twenty-one days after delivery, surviving pups in the F_{1b} litters were weighed and sexed. 20 Pups/sex/level were then representatively selected to initiate the second feeding/reproduction period to produce F_{2a} and F_{2b} litters. Similarly F_{3a} and F_{3b} litters were produced. Twenty-one days after delivery of the F_{3b} litters, 10 male and 10 female weanlings were sacrificed and subjected to gross and histopathological examinations. During the study diet consumption and body weight data were taken, and values for food efficiency and daily intake were calculated. Rats were examined at least once daily for abnormal behavior, mortalities and clinical signs of toxicity. The following tissues from the F_{3b} rats/sex/level were examined histopathologically: brain, adrenals, spinal cord (cervical, thoracic, lumbar, sacral), pancreas, lungs, trachea, thyroid and parathyroid glands, heart, skeletal muscle, sciatic nerve, spleen, thymus, liver, kidneys, testes (with epididymides), ovaries, uterus, stomach, duodenum, jejunum, ileum, colon and bone marrow.