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

OCT 29 1981

MEMORANDUM

OFFICE OF
PESTICIDES AND TOXIC SUBSTANCES

DATE: September 23, 1981

SUBJECT: Thidiazuron (SN49537); Review of validated final report of I.B.T. No. 8533-09630; Three-Generation Reproduction Study in Albino Rats CASWELL #659A

FROM: William Dykstra, Toxicologist
Toxicology Branch/HED (TS-769)

TO: Larry Chitlik, Section Head
Toxicology Branch/HED (TS-769)

Richard Mountfort (23)
Registration Division (TS-767)

for WDD
9/23/81

Recommendations:

1. The sponsor provided additional information in a supplemental report (1/21/81) and a rebuttal statement (4/14/81) that pertained to the deficiencies noted in the Agency validation of 9/10/80. EPL performed an addendum validation (accepted by the Agency 9/23/81) and changed the report from supplementary to valid status based upon this new information and an evaluation of the report was therefore undertaken. The NOEL for the reproduction study is considered to be 200 ppm. The study is acceptable as Core-Minimum Data.

Review:

1. Final Report to Schering AG; Three-Generation Reproduction Study with Thidiazuron (SN49537) in Albino Rats (I.B.T. No. 8533-09630).

   Test Material: SN49537; 100% technical; Batch No. 251120, 1B00000, 271006

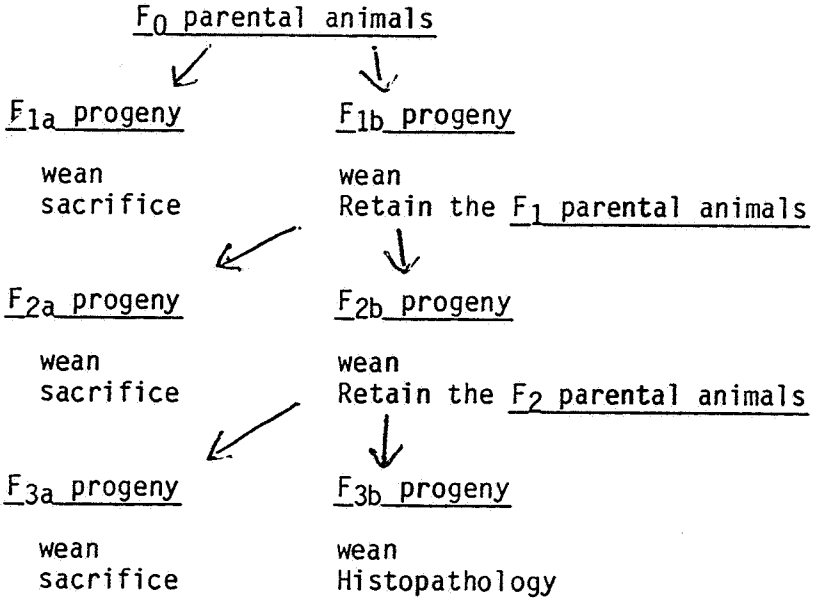
   The animals employed were the Charles River CD strain albino rats. Weanling rats were obtained to form 3 test groups and a control group of F_0 generation animals. The outline of the experiment is shown below:

Group	Dietary Level (ppm)	Number of Animals Males	Females
Control	0	8	16
T-I	60	8	16
T-II	200	8	16
T-III	600	8	16

Parental animals were allowed to reach maturity, mate and produce 2 litters. Eight males and 16 females from the second litters of each dietary group were retained at weaning as parental animals for the succeeding generation. The study terminated following the weaning of the F_{3b} litters. The following lists the duration of each parental generation:

F_0 generation - started 9/14/76, terminated 4/4/77
F_1 generation - started 3/9/77, terminated 10/25/77
F_2 generation - started 8/19/77, terminated 5/3/78

A flow chart depicting the 3 generations is shown below:



The diet for each test group was prepared by incorporating a calculated weight of SN 49537 (100% Technical, Batch No. 25120, 1B00000 and 271006) in measured amounts of standard pulverized stock ration, so that the final concentrations of test material in the diets were either 60, 200 or 600 parts per million (ppm). The animals of the control group were fed the basal ration without test material. Animals in all groups were maintained on their respective diets without interruption until their sacrifice, which followed completion of the weaning of their second litters. Feeding was conducted on an ad libitum basis; fresh diets were prepared and offered weekly. Samples of prepared dietary mixtures were retained and periodically supplied to the sponsor for analyses.

Upon arrival at these facilities (22 days of age), F_0 generation animals were culled and assigned to treatment groups. Each animal was permanently identified by ear-punches, and was housed in a hanging grid-bottom cage that was equipped with a color-coded cage and listing the 1) project number, 2) parental generation, 3) test group, 4) animal's sex, 5) animal's number. Zero day body weights were recorded, and each animal was weighed weekly until the mating trials commenced. No additional body weight data were obtained until the sacrifice of the parental generation, at which time final body weights were recorded.

Observations among animals of each generation for mortality and abnormal behavioral reactions were made daily. In addition, these animals were observed for fertility, length of gestation, and lactation performance.

Three parameters of fertility were calculated: 1) the fecundity index, 2) the male fertility index, 3) the female fertility index. The fecundity index expresses the percentage of copulations which result in pregnancy. This parameter of fertility includes all copulations noted during the mating trials. Male and female fertility indices were then calculated to isolate potential impaired fertility among the sexes. The male fertility index includes only males which had an opportunity to impregnate a fertilite female during the mating trial. The female fertility index expresses the percentage of pregnancies which result from dams having been paired with fertile males.

Mating trials were initiated when the parental animals were 100 days old. Females were caged in pairs and mated with a male from within the same treatment group. Daily examinations were conducted to determine if copulation had occurred. Males were rotated within their dietary group at 10-day intervals until conception was confirmed or until each female had been paired with a maximum of 3 males.

The first litters obtained were weaned and discarded at 21 days post-partum. The parental females were given a 10-day rest period and again mated, the above procedure being repeated in order to obtain second litters. Females failing to become pregnant during the first mating trials were not rebred.

All pups were examined for physical abnormalities at birth and the numbers of viable and stillborn members of each litter were recorded. Records of survival at designated intervals during the lactation period were maintained. Litters of more than 10 pups were arbitrarily reduced to that number on the fourth day of lactation period.

At weaning (21 days post-partum), each pup was again examined for physical anomalies, and the sex and body weight of each weanling was determined and recorded. The weanlings obtained from the first litters produced by each generation of parental animals were sacrificed and discarded. Eight males and 16 females were retained at weaning from the second litters produced to serve as parental animals for the succeeding generation. When possible, litter mates were kept to replace a selected weanling should it succumb within 2 weeks of weaning. Weanlings not retained as parental animals were sacrificed and discarded.

In the event of a parental animal's death, a gross necropsy was performed. Representative tissues and organs were taken for microscopic study in the absence of advanced postmortem autolysis.

Following completion of the second litters, all males and 8 females from each group were weighed, sacrificed, and subjected to gross pathologic examination. At the time of sacrifice, the animals were rendered unconscious with carbon dioxide, and immediately exsanguinated. The weights of the liver, kidney, spleen, gonads, heart, and brain were recorded at the time of necropsy, and a complete set of tissues were retained in 10% formalin for each sacrificed animal.

Complete microscopic pathologic studies were completed upon 5 males and 5 females from the F_0 generation control and 600 ppm test groups. Histopathologic study of the F_1 and F_2 animal's tissue was not conducted by the laboratory, but was conducted by Dr. W.E. Field, pathologist.

Gross pathologic examinations were conducted upon 10 male and 10 female F_{3b} weanlings of each group as described above. Histopathologic studies of these tissues were not conducted by the laboratory.*

The following F_0 generation animals' tissues and organs were examined.**

*Histopathologic examination of the tissues of the control and T-III was conducted by Dr. William E. Field, pathologist.

**The organs and tissues (listed) from F_1, F_2 and F_{3b} were examined grossly by the laboratory and were examined histologically of the control and T-III by Dr. William E. Field.

Tissues and Organs
Examined Grossly

Adrenals
Aorta
Bone
Brain
Esophagus
Eyes
Heart
Intestinal tract
Kidneys
Liver
Lungs
Lymph nodes
Optic nerves
Ovaries
Pancreas
Peripheral nerve
Pituitary gland
Prostate
Salivary gland
Seminal vesicles
Skeletal muscle
Spinal cord
Spleen
Stomach
Testes
Thymus
Thyroid glands
Trachea
Urinary bladder
Uterus

Tissues and Organs
Examined Microscopically

Adrenal gland
Bone marrow (sterum and femur)
Brain (cerebrum, cerebellum and pons)
Caecum
Colon
Esophagus
Heart (right and left ventricles)
Kidney
Liver
Lungs
Lymph node (cervical and mesenteric)
Ovary
Pancreas
Parathyroid gland
Peripheral nerve (sciatic)
Pituitary gland
Prostate
Salivary gland (submaxillary)
Seminal vesicles
Small intestine (duodenum, jejunum and ileum)
Spinal cord (three levels)
Spleen
Stomach (cardiac, fundic and pyloric regions)
Testes
Thyroid gland
Trachea
Urinary bladder
Uterus

Selected tissues and organs were examined upon postmortem animals and those of the intermediate feeding levels which exhibited gross pathologic changes at sacrifice (for the F_0 generation).

All population data and body weight data were analyzed statistically, first, employing a One-Way Analysis of Variance with significant effects disclosed by that treatment further studied by Multiple Comparison.

Statistical analyses were conducted upon the absolute organ weights, and upon the corresponding organ to body weight ratios and organ to brain weight ratios. A One-Way Analysis of Variance was conducted upon the absolute organ weights, and significant effects disclosed by that treatment were further studied by multiple comparison. The organ to body and brain weight ratios were studied statistically employing Kruskal-Wallis statistical analysis first, with significant effects disclosed by that treatment further studied by Kruskal-Wallis multiple comparison.

Results:

Animals fed 600 ppm SN 49537 displayed reduced body weights during the F_2 parental generation (beginning at the initiation of the pre-mating period and continuing to week 9 - males, week 6 - females). These reduced body weights are suggestive of a treatment-related effect.

Behavior, survival and reproductive performance of animals fed SN 49537 were considered to be unaffected by dietary exposure.

Progeny population and survival revealed no consistent intergroup differences which were considered to be the result of SN 49537 exposure. Various intergroup differences were noted for test and control group progeny body weights at weaning. However, these differences were not suggestive of treatment-related findings.

No untoward behavior or reactions were noted among the litters of SN 49537 exposed progeny which were considered to be related to the test material. A single 600 ppm group F_{3a} litter of 6 stillborn pups, 4 of which displayed major developmental anomalies was obtained. No other pups were delivered by test or control dams which displayed structural anomalies when examined following parturition. Various numbers of test and control runt pups, and pups displaying hematomas were noted, though no apparent correlation between the incidences of these observations and the exposure to SN 49537 was observed.

Parental organ weights (obtained at the necropsy of each parental generation) and calculated organ weight ratios revealed no consistent intergroup differences. Gross pathologic studies conducted upon each generation of parental animals (sacrificed and postmortem) revealed no gross pathologic lesions ascribable to the ingestion of SN 49537. Histopathological studies were conducted by the laboratory upon the tissues of the F_0 generation animals only. No treatment-related changes were noted during the histopathological examination of the sacrificed and postmortem F_0 generation animals, and no neoplasms were observed among either the test or control animals.

Gross pathologic studies conducted upon test (all exposure levels) and control progeny of tnis investigation revealed no findings attributable to the SN 49537 exposure.

Histopathologic examination of the organs and tissues of the control and T-III of F_0, F_1 and F_2 parental animals and of F_{3b} weanlings did not reveal any damage which could be attributed to the administration of the compound. Therefore, there is no need to examine the T-I and T-II groups.

Samples of the dietary mixtures employed during this investigation were supplied periodically to NOR-AM Agricultural Products, Inc. for analyses. NOR-AM informed the laboratory that "the results of the analyses showed that the compound was present in the diets at the concentration specified in the protocol (within the limits of experimental error of $\pm$ 100%)."

Conclusion:

The NOEL for the reproduction study is considered to be 200 ppm. The LEL is 600 ppm and the effect was reduced parental body weight during the F_2 generation.

Classification: Core-Minimum Data

TS-769:th:TOX/HED:WDykstra:9-21-81:card 6