From: Reg. No. 201-401, 7/20/89, A. Konitski

7-20-89
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MRID 00093665
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Subject: Two Year Chronic Toxicity Study of S5602 in Rats.

Test Compound: S5602
SD43775
Fenvalerate
Pydrin®

Accession Nos. 246565, -66, -67, -68

Testing Facility: Laboratory Animal Center, Nihon Dobutsu Co., Osaka, Japan. The study was supervised by Sumitomo Chemical Co., Ltd. All examinations were carried out at the Institute for Biological Science, Sumitomo Chemical co., Ltd.

Study No: Document Code AT-10
Ref. No. -0278

Testing Period: Males: February 8, 1977 - February 13, 1979
Females: February 8, 1977 - May 25, 1979.

Report Submitted to Sponsor: April 20, 1981

Purity of Test Material: 93.4%

Lot Number: K-1271

Materials and Methods: Animals: Eight hundred (800) rats of the Wistar/SLC strain from Shizuoka Agricultural Cooperative Association for Laboratory Animals, Shizuoka, were acclimated for one (1) week under animal room conditions controlled for temperature and humidity. Animals were initially housed three per cage but were later changed to 1 animal per cage during the 68th week to prevent the continued transmission of a respiratory disease. Animal Randomization: Animals were shipped sexually segregated, 5-7 rats per box from the breeder. Upon arrival rats from the same box were picked at random and transferred to the first of 27 cages of the prescribed groups (e.g., control group). The first rat selected was placed into the first cage of the (control) group, the second rat picked out of the same box was placed into the second cage of the designated (control) group. This procedure was repeated until every cage of the prescribed (control) group had one rat. This same procedure was then repeated twice so that every cage of the designated (control) group contained three rats each, with the 27th cage containing two rats. The same procedure was followed for the other groups and the other sex. Males and females were kept in separate but adjacent

rooms. Diet Preparation: The control diet was prepared using 500 ml of corn oil (Nisshyoku Co., Ltd., Shizuoka) which was incorporated into 24.5 kg. of the basal diet (CE-2 type, Clea Japan, Inc., Osaka) using a mixer. Diets containing the test article were prepared by dissolving the appropriate amounts of S5602 into 500 ml. of corn oil and mixing with 24.5 kg of the basal diet. Twenty-five kilograms each of 50, 150, 500 and 1500 ppm diets contained 1.25, 3.75, 12.5 and 37.5 grams of test compound, respectively. Fresh diets were prepared weekly. (The diet was shown to be stable for at least one week.) Observations and Measurements: Animals were observed daily for abnormalities and mortality, and were weighed weekly. The amount of food and water consumed for two consecutive days of a week was measured on a per cage basis. Urinalysis examinations were conducted on eight rats of each sex from the dosed groups and the control group after 24 months of feeding. Urine was examined for ketones, occult blood, sugar, protein, pH (Labstix®), bilirubin (Ictostix®), urobilinogen (Urobilistix®) and urine volume. The eyes were also examined. The cornea, iris, lens and retina were examined using an ophthalmoscope at the termination of feeding. Hematology: Hematology was conducted at the termination of feeding. Animals were fasted over night and anesthetized

with diethyl ether. Blood samples were taken from the abdominal aorta. Parameters examined were: erythrocytes, leucocytes, thrombocytes, hemoglobin, hematocrit, mean corpuscular volume (MCV), specific gravity and white blood cell differential count. Clinical Blood Chemistry: Blood serum was analyzed for the following parameters: total protein, albumin, glucose, urea-nitrogen, uric acid, cholesterol, albumin to globulin ratio, sodium, potassium, calcium, GPT, GOT, cholinesterase, leucine aminopeptidase, LDH, AP, creatinine, and creatinine phosphokinase. Pathology: Immediately after the blood had been collected, organs and tissues were grossly examined and dissected out. The following organs were weighed and the ratio of the organ weight to body weight was calculated for brain, lung, heart, liver, kidneys, spleen, testes/ovaries, pituitary, thyroids and adrenals. Histopathology was conducted on the following:

brain	tongue
lung	esophagus
heart	stomach
liver	intestine
kidneys	small
spleen	large

testes/ovaries
pituitary
thyroids
adrenals
eye
spinal cord
sciatic nerve
bone marrow (femur)
lymph nodes
mandibular
mesenteric
skin
mammary gland (females only)
and any tissue appearing abnormal.

salivary gland
pancreas
urinary bladder
trachea
epididymis
prostate
seminal vesicles
peputial gland
uterus
parathyroid

The tissues and organs were preserved in 10% formol-saline (the eye was observed in Bouin's fixative), embedded in paraffin wax, sectioned, stained with H and E and examined under a light microscope. The sciatic nerve was also stained with luxol fast blue and separately with silver (i.e., silver impregnation). A statistical analysis was conducted using the student's "t-test" for body weight, body weight gain, hematology, blood biochemistry, urine volume, and organ weight

and its ratio to body weight. The Mann-Whitney U-test was employed for urinalysis data.

Results

Mortality: The cumulative mortality at temination in male and female animals resulting from spontaneous deaths and those sacrificed in a moribund state were as follows:

	Mortality (%)	
Dose (ppm)	Males	Females
0	78	66
50	75	80
150	72	72
500	64	71
1500	72	71

It was also noted that the cumulative absolute numbers of spontaneous deaths and those sacrificed in a moribund condition from week 64 thru 68 inclusive for males was as as follows:

	Week (Males)	
Dose (ppm)	64	68
0	12 (+38)	50
50	10 (+30)	40
150	13 (+24)	37
500	4 (+17)	21
1500	5 (+23)	28

This was the only observed time period when the death rate for males sharply increased. The death rate for all other time periods remained relatively constant within and between groups. This sharply increased death rate was later shown to be caused by respiratory disease (see discussion).

The death rate for treated females paralleled the death rate for female controls at all time periods. There was no period of time when females showed an accelerated death rate.

Body weights: Males. The initial (pre-dose) weights for the 50, 500 and 1500 ppm dose groups were statistically significantly lower than control weights. The body weight value for the 150 ppm dose group was comparable to the control

group.

The low dose group showed alternating periods of statistically significant and non-significant decreases of body weight. The 150 ppm dose group showed a statistically significant decrease only for about the first half of the study. The high mid-dose group of 500 ppm showed a statistically significant decrease only for the first and last 30 weeks. The high dose group showed a statistically significant body weight decrease for the entire 104 weeks when compared to controls.

Beginning at about week 64, sharp, and parallel body weight decreases were recorded for 4 consecutive weeks in the control group and the two high dose groups. A 4-week recovery period followed (weeks 68-72) which was in turn followed by a steady divergence of the two high dose groups from controls.

Females: Initial (pre-dosing) body weights for females were statistically significantly increased above controls for the low (50 ppm) and high (1500 ppm) dose groups and comparable to controls for the middle dose groups (150 and 500 ppm).

Body weight was decreased in the low dose group after week 70 to termination. Females receiving 150 ppm showed body weight decreases which were statistically significantly lower after week 30 thru termination of the experiment. Animals receiving doses of 500 and 1500 ppm showed statistically significant decreases for the entire experimental duration.

Weight Gain: The final weight gain values for males revealed statistically significant decreases for the following treated groups 50, 500 and 1500 ppm. Final weight gain for the 150 ppm group was comparable to controls. The final recorded weight gain for females showed a statistically significant decrease at the 1500 ppm dose label. Weight gains were, however, comparable to control values at 50, 150, and 500 ppm when analyzed statistically.

Food Consumption: Males: Food consumption for males was generally comparable to controls or sporadically higher than controls. Females showed comparable food intake with controls at all dose levels with the exception of the high dose and high mid dose. Females fed 1500 ppm generally showed raised or statistically significant food consumption increases for the entire experiment, whereas the high-mid dose showed a

raised food consumption.

The average amount of food consumed per day on a gram per kilogram basis appeared to be comparable to controls for both sexes with the exception of the 500 and 1500 ppm dose levels in females where values were two and five grams/kg/day higher than controls.

Water Intake: Water intake was generally comparable between control groups and treated groups.

Clinical Chemistry: Total Protein. Males showed a statistically significant and dose responsive decrease at 1500 ppm. Values for females were comparable to control mean. Albumin: Glucose: BUN: Values in treated animals were comparable to controls for these parameters. Uric Acid: Values for uric acid were statistically significantly decreased from control mean for males at all dose levels. However, values between treated groups were similar and probably were only significant compared to controls in light of the high control value. The values for females were viewed as being comparable to control mean. Cholesterol values were erratic for both males and females. Values at 500 and 1500 ppm for

females were statistically significantly lower but no log dose response was evident. Values for males were comparable to controls. The following parameters revealed no biologically meaningful changes: albumin/globulin ratio, alkaline phosphatase, SGPT, serum cholinesterase, leucine amino peptidase, creatinine, sodium, potassium and calcium. Values in females for SGOT levels were comparable to controls. Values for males were statistically significantly decreased at 50, 150, and 500 ppm, but not at 1500 ppm. No log dose response appeared evident at the three lower dose levels. LDH values were comparable to controls for females and LDH values for males were statistically significantly increased at 150 and 1500 ppm.

Creatinine phosphokinase: Values for males, although statistically significantly lower for all dose levels, were not log dose responsive. The control value appeared to be unusually high. Values for females were comparable to controls.

Hemotology: Values reported for erythrocytes, thrombocytes, hemoglobin, hematocrit, mean corpuscular volume, blood specific gravity, leucocytes and leucocyte differential

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count were all comparable to control values for both sexes with the exception of the male neutrophil count.

Value at 50, 150 and 500 ppm were statistically significantly higher than control values but were not log dose responsive. The value reported for the high dose (1500 ppm) was statistically significantly increased and appeared to be dose responsive.

Urinalysis values for volume, pH, ketone bodies, occult blood, glucose, bilirubin, protein and urobilinogen were comparable to control values.

Absolute Organ Weight: Absolute organ weights for males and females for brain, lung, spleen, ovaries (females), pituitary and adrenals were comparable between treated and control groups. Values for heart in males were statistically significantly lower than controls at 50, 500 and 1500 ppm. However, values were irregular and did not appear to be log dose responsive. Only the high dose female group showed a statistically significant decrease which may not have been compound related. Liver weights for males and females were statistically significantly lower at 150, 500, and 1500 ppm.

A dose response did not appear to be present. Kidney: Kidney weights for males and females showed statistically significant decreases. However, no log-dose response was evident. Thyroid: Males showed no statistically significant decreases. Females showed statistically significant decreases which may have been dose responsive at 500 and 1500 ppm. Testes: Males showed statistically significant increases at 500 and 1500 ppm. A log-dose response was not, however, readily apparent.

Relative Organ to Body Weight Ratio: No statistically significant changes were observed for the following organs: lung, heart, liver, spleen, pituitary, ovaries, and adrenals. Kidneys: Statistically significant increases were noted only in the males at 500 and 1500 ppm. However, a dose response was not readily evident. Brain Weight: Statistically significant increases in brain weight were observed at 1500 ppm for males and females and at 500 ppm for males. However, in males the control value appeared to be low and no dose response appeared evident. In females the increase in the high dose may have been compound related. Thyroid: Values for females were comparable to controls. Values for males were statistically significantly increased at 500 and 1500

ppm. Testes: Values were statistically significantly increased at 500 and 1500 ppm. However, a log-dose response was not readily evident as the value for both dose levels was 1.33.

Pathology: Gross and microscopic observations were generally comparable to controls with the exception of two (2) histopathological findings.

- Compound related giant-cell infiltration of the spleen, lymph nodes, liver and adrenals at dose levels of 500 and 1500 ppm.

- Reticuloendothelial cell proliferation of the mesenteric lymph node. Dose related effects at 500 and 1500.

The above noted observations are considered to be granulomatous changes.

The second noteworthy finding was the increased incidence of interstitial tumors as well as the presence of hyperplasia of the interstitial cells. These summary incidence findings are recorded in the two tables which follow this paragraph.

Summary Incidence of Interstitial Cell Tumors and Hyperplasia of Interstitial Cells

Dose (ppm) / Findings	0	50	150	500	1500
Interstitial Cell Tumors	21	36	27	56	53
Hyperplasia of the Interstitial Cells	19	12	7	10	3
Total	40	48	34	66	56

Summary Incidence of Interstitial Cell Tumors Final Sacrifice and Intercurrent Deaths

Dose (ppm)	Control	50	150	500	1500
Final Sacrifice	6/17 (35%)	14/20* (70%)	9/22 (41%)	28/29** (97%)	19/22** (86%)
Dead and Moribund Sacrificed	15/57 (26%)	22/56 (39%)	18/56 (32%)	28/46** (61%)	34/55** (62%)
Total	21/74 (28%)	36/74* (47%)	27/78 (35%)	56/75** (75%)	53/77** (69%)

*p <0.05 **p <0.01 (x^2 test)

Discussion: Cumulative mortality for males and females between treated groups and between treated groups and controls was comparable. Additionally, the rate of dying was generally parallel between all groups within sexes. Males showed a short period of accelerated death rate between weeks 64 thru 68. This accelerated rate of dying was attributed to a respiratory infection. Although animals died in all groups, most deaths occurred in the control group with a decrease in the number of deaths with increased dose. The number of animals dying per dose group during this period was argued to

be the result of cage placement in the room. This event will be expanded upon in the discussion of the significance of testicular tumors in males.

Food consumption for males was comparable for all groups and final body weight gain seemed to reflect initial body weights. Initial body weights were statistically significantly lower than controls at initiation for 50, 500 and 1500 ppm dose groups and final body weight gains were statistically significantly lower at 50, 500 and 1500 ppm. However, during the course of the experiment in the 1500 and 500 ppm dose group body weights were statistically significantly decreased for 104 weeks and the last 30 weeks, respectively, when compared to controls. The duration and the degree of effect at 1500 ppm might be considered compound related whereas the effect at 500 ppm may be open to interpretation. However, due to the lack of randomization of the animals (to be expanded on later) this conclusion is not considered definitive for males. Food consumption in the high dose female group and the high mid-dose female group was much higher than controls. Increased food consumption in the presence of statistically significantly decreased body weight gain during the experimental duration for the two high dose groups and comparable food

consumption with decreased body weight in the two low dose groups seems to argue for effects at all dose levels in females. It is reiterated here that the initial body weights for females were statistically significantly increased above controls for the 50 and 1500 ppm dose groups and comparable to controls for the middle dose groups (150, 500 ppm). The decreased body weight in females therefore appears to be compound related. The Japanese authors indicated that "lower body weight gain in 500 and 1500 ppm males and 1500 ppm females and slightly higher adjusted food consumption in 1500 ppm females might be caused by the compound." TB is in basic agreement with the authors except for the association of the sex with the body weight and doses. It is our opinion that the company report consists of transposing errors (or a translation error).

The Japanese authors indicated that there were no compound related changes in mortality, water intake, blood chemistry, urinalysis, ophthalmology and gross pathology. We in our review generally agree with these conclusions. Examination of these data indicate effects which are not biologically meaningful either because the events are singular, not log-dose responsive, not supported by confirmation data within

within the experiment, or that reported values are in contrast to normal expectations of toxic responses.

The authors also indicate that the slightly higher neutrophil counts in the 1500 ppm male dose group might be compound related. TB agrees with this statement.

Non-neoplastic changes observed during histopathological examination revealed giant cell infiltration of the spleen, liver, lymph nodes and adrenals at a dose of 1500 ppm. Giant-cell infiltration was also noted in lymph nodes and adrenals at 500 ppm. Dose related increases of reticuloendothelial cell proliferation were noted in the mesenteric lymph nodes at 500, and 1500 ppm. These non-neoplastic changes of giant cell infiltration and reticuloendothelial cell proliferation have been observed in other company submitted studies and are <u>not</u> considered as "new findings" by this reviewer. These granulomatous changes are considered responses to foreign substances. Other non-neoplastic changes were not considered to be compound related.

Testicular tumors (testicular interstitial cell tumors) were observed in all groups including controls. The tumor

incidence appeared to increase with dose with the maximum responses recorded in the two high dose groups. The study sponsors therefore presented arguments that the increased incidence was not considered to be compound related. The following points were made by the sponsor in support of their position:

1. Tissue Typing and Historical Testicular Tumor Incidence of the Japanese Wistar/SLC Rat.

"High incidences of testicular tumors have not been reported in typical Wistar rat strains (references were included). Hcwever, a highly variable incidence (11% to 100%) of spontaneously occurring tesicular interstitial cell tumors (TICT) in control Wistar/SLC rats from the supplier used in this study has been observed in the last six years (see attached table No. 4 Incidences of Naturally Occurring Intestitial Cell Tumors in Wistar/SLC Rat* (By Literature Survey) ...tissue typing of the Wistar/SLC strain was performed to identity its hereditary relationship to Fischer 344 (inbred) and two other Wistar rat strains. Tissues typing showed that the Wistar/SLC strain to

be genetically different from two other Japanese Wistar rat strains [Wistar/JCL (closed colony) and Wistar/Imamichi (closed colony)]. More importantly the Wistar/SLC strain was shown to be genetically very similar to the Fischer 344 strain. Fischer 344 strain rats are known for their high incidences of spontaneous testicular tumors while Sprague-Dawley and Wistar rat strains are known for their low incidences of this tumor" (reference provided in the report).

"...The animals placed on this study possessed a genetic predisposition to the development of a high and/or variable incidence of spontaneously occurring testicular tumors."

2. Effect of Non-Random Allocation of Litter Mates to Treatment Groups.

Rats were not distributged in a truly random manner between groups. Rats were distributed among groups in the following non-random manner.

"...five to seven rats of the same sex arrived in shipping boxes from the supplier. One rat was picked out and transferred to the first of 27 cages of the prescribed groups, e.g., control or any one of the dose groups. The next rat taken out of the shipping box was placed in the next cage of the same group and so on until the shipping box was empty. After removing the last rat from the shipping box the same procedure was repeated until each cage contained three rats (except every 27th cage which contained two rats). According to the supplier, the animal shipping cartons very often contain litter mates. The use of this non-random cage assignment procedure likely resulted in litter mates being allocated to the same treatment group. This grouping procedure obviously biased the animal, the animal distribution and likely biased development and distribution of spontaneous testicular tumors in control and treatment groups.

"As mentioned previously, there was a highly variable incidence of testicular tumors among

the different shipments of Wistar/SLC rats from this supplier during this six-year period. Studies conducted within the same relative time frame, albeit not at the same facility, using rats from the same "homogenous gene pool" showed a 16-100% range of testicular tumor incidence at 24 months. This raises serious questions of the degree of heterogeneity of this Wistar/SLC gene pool. This Wistar/SLC strain may not be highly inbred, and in fact may have been cross-bred with Fisher 344. If so, the incidence of spontaneous testicular tumors would be directly proportional to the specific genetic components of the individual breeder pairs and the specific time period in which these rats were used as breeders. When the spontaneous incidence of testicular tumors within a specific strain is so variable, the importance of an unbiased animal randomization procedure becomes critical or the recognition of an induced tumor response is seriously compromised."

3. Effects of Disproportionate Mortality and Non-Uniform Distribution of Male Rats in a Female Environment.

"Until week 68, male and female animals were housed three to a cage with males and females housed in separate but adjacent rooms. Information on the animal rooms, cage distribution and individual animal groupings were presented in an attachment." During weeks 64-68 there was an apparent dose-related occurrence of male mortality (i.e., 48,[sic] 30, 24, 17 and 24 for 0, 50, 150, 500 and 1500 ppm treatment groups, respectively. Note: the value of 48 is in error and should be 38). This was caused by a viral respiratory disease that was spread rapidly through the males from the entry door inward. (Note: the high dose was near the door with the progressive lower doses in sequence, toward the rear; the air flow was from the doorway to the rear of the room.) The male mortality reduced the numbers of control and low dose male rats at risk of developing testicular tumors, e.g., 19 of the control rats that died during the disease outbreak had already developed testicular hyperplasia. This disproprotionate mortality in

rats genetically predisposed to spontaneously developing testicular tumors had an effect upon the overall testicular tumor incidence, but the degree to which it was affected cannot be accurately assessed due to the initial non-random allocation procedure employed.

"During week 68, all rats were individually housed and redistributed among the cage racks in the two (2) animal rooms (diagram of the distribution was provided). This animal redistribution drastically altered the environmental status of the study in that male rats were introduced into a female environment. There are numerous reports that in many species proximity to females (without physical contact) can affect the reproductive system of the male rat. Close proximity of the male rat to the female results in increases in luteinizing hormone (LH) and pituitary and plasma testosterone levels. This is thought to be a male endocrine response to the odor of female urine. It is also known that the growth and development of testicular tumors are stimulated by hormonal changes in the aging rat.

In this study there was a statistically significant increase in the average number of females surrounding male rats in a doserelated fashion due to the disparate male mortality. Whether the apparent dose-related increase in testicular tumors was related to the dose-related greater number of female rats surrounding a male rat is not known. However the increased numerical ratio of female to male rats may have contributed to the incidence of testicular tumors."

The average number of female rats surrounding a male rat after 68 weeks was as follows:

Dose Group (ppm)	Average No. of Female Rats Which Surrounded a Male Rat
Control	1.00+/-0.00
50	1.38+/-0.08*
150	1.67+/-0.11*
500	1.76+/-0.06*
1500	2.27+/-0.09*

*p <0.01 (u-test)

Conclusion:

- The lower body weight gain in 500 and 1500 ppm dosed females concurrent with increased food consumption is suggestive of a compound related effect in females. The non-random allocation of females resulted in

higher and/or comparable body weights for groups to be treated with compound when compared to control groups. Females at termination had lower body weight than controls accompanied by increased food consumption.

- The lower body weight gain in males at 1500 ppm appears to be compound related. However, due to the non-random allocation of males between groups, the low, mid-high and high dose groups were statistically significantly lower than controls at initiation. The high-dose group began and ended the experimental below control values. However, the duration of body weight depression (104 weeks) seemed to suggest a compound related effect when the effect was compared to controls and other treatment groups. However, due to the lack of randomization of the animals this conclusion cannot be considered definitive.

- Higher neutrophil counts in the 1500 ppm male dose group might be compound related.

- Giant cell infiltration was observed in lymph nodes

and adrenals at 500 and 1500 ppm of both sexes and at 1500 in spleen and liver. Dose related increases of reticuloendothelial cell proliferation were noted in the mesenteric lymph nodes at 500 and 1500 ppm. The effects have been previously observed in other studies.

- Interstitial cell testicular tumors were not considered to be compound related for the following reasons:

  - Tissue typing of Wistar/SLC strain rats have shown them to be genetically similar to Fischer 344 strain rats. Fischer 344 strain rats have a high occurrence of sponaneously occurring testicular tumors.

- Historical testicular tumor incidence of Wistar/SLC strain rats shows a highly variable spontaneously occurring tumor incidence. This implication is one of a "non-homogeneous gene pool" for Wistar/SLC strain rats.

- Non-random allocation of animals, as noted in the protocol and generally supported by the evidence of

non-uniform body weight distribution into treatment groups, combined with the real probability of litter mate distribution into the same treatment groups, resulted in some non-definitive conclusions for chronic effects, and cast sufficient doubts on the interpretation of the appearance of interstitial cell testicular tumors as to invalidate the oncogenicity portion of this study on this point alone.

- The disease related mortality in males reduced the number of animals at risk in developing interstitial cell testicular tumors in the controls and low dose groups. However, it was pointed out that many of the controls and low dose animals manifested hyperplasia of the interstitial cell tissue of the testicles. The degree to which overall testicular tumor incidence would have occurred had the disease outbreak not occurred is not assessable.

- The redistribution of males and the interspersion of males among females introduced into the experiment an uncontrolled variable and the arguable point of increased luteinizing hormone levels in males and

plasma testosterone levels, caused by the female presence, which may have contributed or influenced the incidence of testicular tumors.

Overall this reviewer agrees with the position and arguments presented by the sponsor that "the many uncontrolled factors and events that occurred during the study so complicated evaluation of the data obtained that the significance of the testicular tumors is rendered uninterpretable," and classifies the oncogenicity portion of this study as invalid and classifies the chronic feeding aspects of this study as core-supplementary. This study does not have to be re-run. It is also pointed out to the reader that the sponsor has previously submitted an oncogenicity study in Sprague-Dawley rats tested at 1000 ppm. The results were negative for oncogenicity and the study classified as Core-Guidelines. Additionally, oncogenicity studies with mice were also negative and classified as core-guideline.