Reading

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

JUL 20 1984

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MEMORANDUM

OFFICE OF
PESTICIDES AND TOXIC SUBSTANCES

SUBJECT: Pydrin EPA Registration No. 201-401. Miscellaneous Data. Accession No. 246560-246568 Inclusive. (Includes Six Month Dog Study and Change of Species for Calculating the PADI)

FROM: Albin B. Kocialski, Ph.D.
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Hazard Evaluation Division (TS-769C)

Albin B Kocialski 7/19/84

THRU: William L. Burnam, Chief
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Hazard Evaluation Division (TS-769C)

W L Burnam 7.20.84

TO: Timothy Gardner, PM #17
Registration Division (TS-767C)

TOX Chem. No. 77A

The Shell Chemical Company has submitted several studies for toxicological review. These studies were as follows:

Study 1. A six month dietary feeding study in dogs with techical SD-43775. (Core-Guideline; study required for registration. A NOEL was not established.)

Study 2. Life span chronic toxicity study of S5602 in mice (ddy strain). (Core-Guideline; this study not required for registration; a previous study was submitted and accepted.)

Study 3. Reversibility of granulomatous changes in ddy strain mice fed S5602. (Core-Supplementary; this study not required for registration.)

Study 4. Two year chronic toxicity study of S5602 in rats. (Core-Supplementary for chronic aspects; Invalid for Oncogenicity; this study not required for registration; a previous study was submitted and accepted.)

Study 5. Hereditary relation among Fischer 344, Wistar/SLC, Wistar/JCL, Wistar/Imamichi and CD(SD) rats. (No Core classification but determined to be Acceptable; this study not required for registration.)

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Study 6. Neurotoxicity study. The effects of 20 oral doses of fenvalerate (S5602) over a period of 4 weeks on the rat sciatic and posterior tibial nerves and trigeminal ganglion. (Core-Supplementary; this study not required for registration.)

Study 7. Species susceptibility of Pydrin insecticide in the rat, mouse, hamster and rabbit. (Core-Supplementary ; this study not required for registration.)

Study 1.

A review of the six (6) month dog study has led to the conclusion that a a NOEL has not been established. The lowest effect level has resulted in the following effects; emesis, headshaking, biting of the extremities, normocytic anemia, increased serum cholesterol levels, possible CNS and peripheral nerve dysfunction and hepatic microgranulomatosis. It is necessary that this study be reconducted. (NOTE: Shell Chemical Company at a previously held joint meeting between EPA representatives and the Shell Chemical Company has agreed to conduct a new six month dog study. This new study will however be conducted on their new "enriched" technical. This new technical will consist of only two (2) of the four (4) optical isomers currently present in the technical. One of the two isomers in this "enriched" technical will be the pesticidally active isomer which was also present in the "old, non-enriched technical." It is noted that of the four (4) optically active isomers only one (1) is pesticidally active. This new study will be used to support the registration of Pydrin (fenvalerate) and satify the data gap for the non-rodent chronic toxicity study). (See also page 5; change in calculating the ADI.)

Study 2.

The life span chronic toxicity study of S5602 in mice was conducted and a NOEL of 30 ppm was established. The LEL was 100 ppm based on findings of granulomatous changes in males and females. Granulomatous changes were observed at 100 and 300 ppm for liver and lymph nodes and at 300 ppm for spleen. Slight but a statistically significant decrease in RBC count was accompanied by a statistically significant increase in MVC in males at 100 and 300 ppm.

Study 3.

A study was conducted to determine the reversibility of granulomatous changes in ddy strain mice for liver, spleen and mesenteric lymph nodes. Some reversibility of liver cell granulomas as well as giant cell infiltration of the mesenteric lymph nodes was observed. However, within the time frame of the experiment the reversibility was not total. Total reversibility was noted in the spleen. (NOTE: The sponsor has indicated in a subsequent meeting that the granulomatous changes were attributable to one (1) of the four (4) optically active isomers found in the technical. The registrant based his position on informational studies conducted in Japan. It was also indicated by the sponsor that the "new-enriched" technical would not contain the isomer which was responsible for these granulomatous changes.

Study 4.

A two-year chronic rat feeding/oncogenicity study was conducted in Japan. The chronic feeding aspect of this study was classified supplementary and the oncogenicity portion of this study was classified invalid. The arguments for invalidating the oncogenicity aspect of the study as presented by the registrant and agreed with by this reviewer and MITRE Corp. (contractor to EPA) were as follows. -- The integrity of the study was compromised and the increased testicular tumor incidence can not be related to treatment because of (1) the highly variable spontaneous testicular intersticial tumor incidence, (2) the non-random allocation of animals into test groups, (3) the development of respiratory disease, and (4) the redistribution of males into a female environment.

Study 5.

The study conducted to determine genetic similarity between the Wistar/SCL strain of rat and other strains of rat including the Fischer 344 rat was conducted to add a supporting argument for discounting the testicular tumors observed in the two year chronic rat feeding study noted in Study 4. The conclusion reached by an independent contractor (MITRE Corp) indicated that the genetic evidence presented by the registrant did confirm the predisposition toward the spontaneous appearance of testicular tumors as seen in the 2-year rat study. The genetic evidence suggested that the Wistar/SCL strain may not be highly inbred, exhibits genetic drift, or that the strain may have been at one time bred to the Fischer 344 strain of rat.

Study 6.

A rat neurotoxicity study was conducted to evaluate the effects of 20 oral doses of fenvalerate over a period of 4 weeks on sciatic and posterior tibial nerves and the trigeminal ganglia. A NOEL of 12.5 mg/kg/day was determined. The LEL for this study was 100.0 mg/kg/day over the 20 day period. Minimal clinical signs were observed. Neuropathology was negative for sciatic and posterior tibial nerves and the trigeminal ganglia. Small increases in the enzyme markers, beta-glucuronidase and beta-galactosidase levels were observed. Males appeared to be more affected than females.

Study 7.

A study was conducted to determine the species susceptibility of fenvalerate using the rat, mouse, hamster and rabbit for comparison purposes. Using AOLD50 and AOED50 (effective dose 50; clinical toxicity signs) determination of species susceptibility to fenvalerate was determined. They were in decreasing order; mice, rats, hamsters and rabbits. The following neuropathological correlations were made with respect to lesions within each species.

° Mice - a statistical correlation between the incidence of clinical signs of toxicity and the incidence of nerve lesions was not observed at doses around the ED50 and LD50.

° Rats - a statistically significant correlation between the incidence of clinical signs of toxicity and the incidence of nerve lesions was not observed at doses around the ED50, although the actual number of rats that displayed both clinical signs of toxicity and nerve lesions tended to increase in some doses around the LD50 the correlation between clinical signs and nerve lesions was reported to be not statistically significant.

° Hamsters - hamsters did not show any correlation between the incidence and severity of clinical signs and the incidence and severity of nerve lesions.

° Rabbits - neurological injuries occurred as a result of convulsions and the rabbit data was therefore not subject to a statistical analysis.

It is pointed out here for the record that the Agency has on file rat and mouse chronic feeding/oncogenicity studies which are used to support the registration of Pydrin (fenvalerate).

Change in Calculating the ADI. Removal of the Rat and Use of the Dog in Determining the ADI. Provisional ADI Based on the Dog Study.

The rat is currently used in calculating the ADI. The NOEL in the rat study was 250 ppm (12.50 mg/kg/day) and translated to an maximum acceptable daily intake of 0.1250 mg/kg/day or an MPI of 7.50 mg/day (60 kg). Since the value obtained thru the dog study is more conservative on a mg/kg basis than the value obtained thru the rat study the ADI (MPI) will therefore be calculated on the value obtained in the dog study. We point out however that the value of 250 ppm was not a no-observable-effect level and can not be considered a definitive NOEL at this time. The value obtained in the dog study is at this time considered only a provisional NOEL.

The Shell Chemical Company has previously responded and addressed those signs and experimental results which negated the establishment of a NOEL in the six month dog study. Toxicology Branch has responded to the registrants arguments and the Toxicology Branch review is attached here for informational purposes. (See review by Marion Copley, Accession No. 250251, TOX Chem. No. 77A, "Discussion Document on Ouestions Raised by EPA on Pydrin® 6 Month Dog Study.") This review is however in response to a separate submission sent in to the Agency.

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Addendum

Study Type: Two Year Chronictoxicity Study in Rats

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

Sponsor: Shell Development Company and Sumitomo Chemical Co. Ltd.

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

Date: April 20, 1981

The Toxicology Branch requested an outside contractor (MITRE Corp) to independently evaluate arguments presented by the sponsor which stated in essence that the integrity of the study was compromised by various factors and rendered the significance of the testicular tumors questionable.

The purpose of the first MITRE review was to determine whether or not the intergrity of the study was compromised by the overall procedures and circumstances occuring during the conduct of the study. The purpose of the second MITRE review was to determine whether or not the SCL strain of Wistar rats used in this study were or were not genetically similar to the Fisher 344 rat and therefore predisposed to the occurance of testicular tumors.

The conclusion reached by the MITRE Corp with respect to the first MITRE Corp review was as follows:

"In summary, because of: (1) the highly variable spontaneous testicular interstital cell tumor incidence; 2) the non-random allocation procedure used; 3) the development of respiratory disease; and 4) the redistribution of males into a female environment, the integrity of this chronic toxicity study was compromised and the increased testicular tumor incidence cannot be related to treatment."

The conclusion reached by the MITRE Corp with respect to the second MITRE Corp review was as follows:

"Wistar/SLC rats treated in a chronic study with S5602/SD43775 at 50, 150, 500, or 1500 ppm in the diet showed statistically significant increases in the incidence of testicular interstitial cell tumors compared to the controls. The biological significant of the increased incidence is moot because of the high and variable spontaneous incidence of these tumors in the SCL strain of rats used in Japan and obtained from one supplier. Tissue typing studies show that the SLC strain resembles the F344 strain more closely than it resembles other conventional strains of Wistar rats and Sprague-Dawley rats for which a low incidence interstitial cell testicular tumors is common (0-5%). The similarity is corroborated by a literature survey of the genetic and biochemical markers of a number of rat strains. The genetic evidence does confirm the predisposition toward the spontaneous appearance of these tumors and suggests that the SCL strain of wistar rats may not be highly inbred, exhibits "genetic drift" or that the strain may have been at one time bred to the Fischer 344 strain."

Conclusion: Independent evaluation of arguments presented by the sponsor confirmed that the integrity of the study was comprised and that the increased testicular tumor incidence could not be related to treatment.

Attachments: 2 MITRE Corp reviews

Subject: A Six Month Dietary Feed Study in Dogs with Technical SD-43775

Test Compound: Technical Fenvalerate
Pydrin®
SD-43775 Technical

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Accession No.: 246562

Testing Facility: Borriston Laboratories, Inc.
Temple Hills, Maryland

Study No.: Borriston Project No. 208-B

Testing Period: August 4, 1980 - February 13, 1981

Report Submitted to Sponsor: May 26, 1981

Purity of Test Material: 91%

Batch or Lot Number: 14200-92-77B

Test Diet Samples: Homogeneity, Stability, Concentration: No data reported for these parameters. Assumption made by the reviewer that each objective for each parameter was attained.

Materials and Methods: Test Article: SD-43775 Technical, an amber-colored liquid, was received from the Shell Chemical Co. by Borriston Labs in six (6) clear glass bottles on June 25, 1980. Upon receipt, the bottles were wrapped in alumimum foil and were stored at room temperature. The purity of the test article was 91% active ingredient and was adjusted to 100% for dosing purposes.

Animals and Husbandry: Twenty-seven male and 27 female beagle dogs were received from Marshall Research Animals (North Rose, New York). The dogs were approximately 4 months of age upon receipt. Body weights for males ranged between 6.1 to 8.5 kg and for females from 5.4 to 7.3 kg. All dogs had been dewormed and vaccinated against rabies and immunized against certain diseases by the supplier.

All animals were maintained in quarantine for six weeks in the environmentally-controlled room in which the study was to be conducted. Animals were housed individually in raised stainless steel grid cages. During the quarantine period Certified Purina® Dog Chow Meal® was offered daily in approximately 350 gram rations. Water was presented in bowls and available ad libitum.

All animals were observed daily for mortality, general physical appearance, behavior and clinical signs. Baseline hematology and clinical chemistry testing was performed on each dog. Additionally, general health determinations were made for each animal and included ophthalmologic and urinalyses, examinations. Individual food consumption and body weights were measured and recorded once weekly for two weeks prior to dosing for baseline determinations.

Twenty-four male and 24 female clinically acceptable dogs were randomly assigned to the study using a computerized randomization procedure. Animals selected possessed similarity of body weight distribution between same-sexed treatment groups. All animals were uniquely identified by ear tag and cage card.

Animals were divided into the following treatment groups:

Group	Dogs	Dosage Level (ppm)
1	6M	0 (control)
2	6F	0 (control)
3	6M	250
4	6F	250
5	6M	500
6	6F	500
7	6M	1000
8	6F	1000

Feed was prepared every two weeks and stored at room temperature in polyethylene bags inserted into air-tight plastic barrels.

Each animal was fed approximately 325 g of the appropriate test or control diet for a 4 hour period on a regular daily schedule 6 days per week. Qualitative food consumption estimates (estimated as percent of food consumed) was recorded on these days. On the remaining day of the week all dogs were offered exactly 325 g of diet during the four-hour period and quantitative food consumption measurements were recorded.

Each dog was observed twice daily for general physical appearance, behavior, pharmacological signs and mortality. Body weights were recorded once a week.

Hematology and Clinical Chemistry analyses were performed at monthly intervals during the course of the study. Blood samples were obtained from each animal by jugular puncture following overnight fasting. Urinalyses were conducted one week prior to dosing and at 2, 4 and 6 months during the study.

The following Clinical Pathology tests were performed:

Hematology

- Packed Cell Volume
- Total Hemoglobin
- Erthrocytes
- Leukocytes
- Differential Leukocyte Counts
- Reticulocytes
- Platelet Count

Clinical Chemistry

- Urea Nitrogen
- Fasting Glucose
- Total Protein
- Albumin
- Globulin
- Sodium
- Potassium
- Phosphorous
- Calcium
- Cholesterol
- Glutamic Oxaloacetic Transaminase
- Pyruvic Transaminase
- Lactic Dehydrogenase
- Alkaline Phosphatase
- Total Bilirubin
- Direct Bilirubin

Urinalysis

- Gross Apperance
- Specific Gravity
- pH
- Qualitative Analysis
  - Albumin
  - Glucose
  - Occult Blood
  - Bilirubin
  - Urobilinogen
  - Ketone
  - Porphobilinogen (at 6 month interval only).
- Microscopic Examination (formed elements).

. Ophthalmologic examinations were performed on all animals prior to treatment and prior to sacrifice. Examinations were performed by a Board-certified veterinary ophthalmologist using a funduscope following pupillary dilatation with a 1% atropine sulfate solution.

Neurological Examination: A neurologic examination of each dog was conducted prior to terminal sacrifice by Dr. Steven Simpson, a consulting veterinary neurologist. A complete neurological examination was performed on at least 6 dogs of each test group. Some dogs were only examined once; others were examined 3 times. The neurological examination consisted of evaluation of each of the following tests: mental status, gait and posture, cranial nerve exams, spinal reflexes, postural reactions and response to sensory stimulation. The number of animals examined in each dose group was as follows:

Dose (ppm)	Males	Females
0	4	2
250	2	4
500	3	3
1000	3	4

A sample evaluation sheet is attached.

All surviving dogs were narcotized with an intravenous dose of sodium pentobarbital and sacrificed by exsanguination. Complete necropsy was performed on each dog under the supervision of a Board-certified veterinary pathologist and all gross lesions recorded. A complete necropsy examination was also performed on the one dog which was sacrificed in extremis during week 25 of the study.

Samples of the humerus bone were taken from each dog, at the time of terminal sacrifice and duplicate slides of marrow prepared for complete microscopic marrow differentiation enumerations (and myeloid/erythroid ratio).

The following organs from all animals were weighed prior to fixation: brain, heart, liver, kidneys, pituitary, thyroid, ovaries/testes and adrenals. Organ/body weight ratios were determined using the fasted terminal body weights which were measured at the time of sacrifice.

All tissues were preserved in 10% neutral buffered formalin with the exception of eyes and testes which were preserved in Bouin's fixative. Following fixation, tissues were processed, embedded into paraffin blocks, sectioned, mounted on glass slides and stained with hematoxylin and eosin.

The following organs and tissues from each dog were examined microscopically by a Board-certified veterinary pathologist:

Brain (3 levels)	Liver (section from each lobe)
Pituitary	Gallbladder
Spinal cord (cervical, thoracic, lumbar)	Kidney
Eyes	Spleen
Salivary gland (parotid)	Mesenteric lymph node
Thyroid (with parathyroids)	Urinary bladder
	Testes/ovaries
Thymus	Corpus and cervix uteri
Heart	Skin (dorsal lumbar)
Aorta	Mammary gland
Tongue (through taste bud)	Skeletal muscle (psoas)
Trachea	Bone marrow (humerus)
Esophagus	Sciatic nerves (proximal and distal)
Stomach (glandular, nonglandular)	Distal tibial nerves
Lungs (with mainstem bronchi)	Distal plantar nerves
Small intestine	Peroneal nerves
Large intestine	Ulnar nerves
Adrenals	Facial nerves
Pancreas	Optic nerves
	All gross lesions

Transverse and longitudinal sections of the tibial and planter nerves and longitudinal and cross sections of all other nerves were examined.

Means and standard deviations were calculated for all body weight, quantitative food consumption, hematology, clinical chemistry and organ weight data, for each control and treated group.

Results: The following treatment related pharmacotoxic signs were observed with respect to appearance and behavior:

- Biting of the extremities
- Head shaking
- Ataxia
- Tremors
- Thin (i.e., animals appearing thin)
- Emesis

Biting of the extremites was observed in both male and female dogs in all treated groups with 5 or more animals being affected at each dose interval. The total number of male animals affected and the cumulative number of observations recorded were substantially raised above control values.

A dose response decrease in onset was noted with increased dose beginning with the low dose tested.

The number of females showing a biting response of the extremities was comparable for all groups. However, the cumulative number of observations recorded was raised above the control value with treated groups showing an increase in incidence beginning with the low dose. A substantial decreased time to onset was also noted with increased dose.

Head shaking was observed in the low, middle and high dose male treated groups with no observations recorded for the control group. The total number of animals affected increased with dose as did the cumulative number of observations recorded. A decreased time to onset was recorded with increased dose.

The number of females manifesting head shaking appeared comparable to the control group. The cumulative number of observations also appeared comparable to controls for the low and mid-dose group. The high-dose group did however show an 18 fold increase in cumulative incidence over the control value. A decreased onset time was also observed with an increased dose beginning at the mid-dose level.

Ataxia was not observed in either the control group or the low dose group for males or females. The total number of animals affected as well as the cumulative incidence was substantially increased over control values for the mid and high dose groups for both sexes. A decreased time to onset was also observed for the mid and high dose groups for both sexes and gave indication of being dose responsive.

Tremors were not observed in male dogs of the control or low dose group. The mid and high dose group of males showed an increase in the number of animals affected as well as in the cumulative number of observations recorded. The time to onset however did increase with increased dose.

The female high dose group was the only group to show a substantial increase in the number of animals affected as well as a substantial increase in the cumulative number of observations for tremors when compared to control values.

Thinness: Treatment related thinness was observed in both the high and low dose treated females. Thinness was not observed in the control group of female beagles. Treatment related thinness was not readily apparent in males.

Emesis was observed in almost all animals of all groups of both sexes. However, only the male high dose group and the female mid and high dose group showed any cumulative incidence which may be compound related. However, time to onset of emesis was substantially decreased in a dose response manner -

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PROJECT 208-B SUMMARY OF TREATMENT RELATED PHARMACOTOXIC SIGNS WITH CUMULATIVE INCIDENCES[a] AND MEDIAN RESPONSE TIMES[b]

SIX-MONTH DIETARY FEEDING STUDY IN DOGS

PHARMACOTOXIC SIGN	MALES 0	MALES 250	MALES 500	MALES 1000	FEMALES 0	FEMALES 250	FEMALES 500	FEMALES 1000
GROUP NO.	1	3	5	7	2	4	6	8
BITING EXTREMITIES								
Number of animals affected	1	5	6	6	5	6	6	6
Cumulative incidence	2	52	113	97	15	109	71	166
Median response time	157	122	16.5	6.5	58	79	15.5	15
HEAD SHAKING								
Number of animals affected	0	2	3	6	4	3	5	6
Cumulative incidence	-	6	6	58	8	3	12	140
Median response time	-	160	23	8	160	185	104	66.5
ATAXIA								
Number of animals affected	0	0	5	6	0	0	6	6
Cumulative incidence	-	-	21	268	-	-	23	425
Median response time	-	-	8	4	-	-	6.5	1.5
TREMORS								
Number of animals affected	0	0	4	6	1	1	2	6
Cumulative incidence	-	-	7	23	2	1	2	70
Median response time (days)	-	-	7	15	10	128	95.5	7
THIN								
Number of animals affected	1	1	2	0	0	3	1	5
Cumulative incidence	19	1	180	-	-	64	5	454
Median response time	180	194	54.5	-	-	33	44	44
EMESIS								
Number of animals affected	5	5	5	6	4	6	5	6
Cumulative incidence	12	8	13	20	13	15	34	23
Median response time	107	31	3	1	23	6.5	5	1.5

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[a] Cumulative incidence is given as the total number of times the sign was observed in each group throughout the treatment period.

[b] Response time is given as the median time (in days) from study initiation to the first observation of the sign for each group.

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Note: A xerox copy of the tabular summary of treatment related phamacotoxic signs with cumulative incidences and median response times is attached. — This is page 8.

Page 8 is the following table. 004041

Mortality: One high dose (Group 7) male was sacrificed in a moribund condition after manifesting sings of hyperactivity, twitching, severe convulsions and finally, unresponsive behavior. During the week prior to sacrifice (week 24) the animal was reported to have exhibited aggressive behavior. No other dogs were sacrificed prior to the terminal kill.

Body Weights: Males: No statistically significant body weight decreases were observed at any dose level for any time period. Females: Statistically significant (Student's t-test at $p < 0.05$) and consistent body weight decreases were recorded for females after the second week of compound administration for those animals receiving the high dose of 1000 ppm. Groups 4 and 6, although not statistically significantly lower, were lower did show dose related decreases for the treatment period.

Food Consumption: Males: No consistent statistically significant decreases were observed for any dose level. Food consumption values were considered to be comparable to control values. Females: Food consumption values were not statistically significant from control values for the low and mid-dose group. Food consumption values for the high dose group were comparable to controls at all time periods with only the following weeks showing statistically significant decreases; weeks 2, 3, 5 and 17.

Hematology: Males: Examination of hematology data revealed statistically significant decreases for RBC at all dose levels. A dose related trend was evident. Statistically significant decreases were observed at 250 ppm for 1, 2 and 4 months, at 500 ppm for 1, 3, 4 and 6 months and at 1000 ppm for 2, 3, 4, 5 and 6 months. Statistically significant decreases for both hemoglobin (Hb) and hematocrit (HCT) were only observed at the high dose level for 2, 3, 4, 5 and 6 months. In those cases where Hb or HCT values were not statistically significantly decreased from controls the values were generally lower than control values.

Females: RBC count was statistically significantly decreased for females at the high dose level at 1, 2, 3, 4, and 5 months. The 6 month value, though lower than control value, was not statistically significant. Hemoglobin and hematocrit values were statistically significantly decreased from 1 through 5 months in the high dose group. Values at 6 months were lower than controls but were not statistically significant. Values for hematocrit, hemoglobin and RBC for the low and mid-dose groups although not statistically significantly lower were generally lower than controls.

Decreases noted in the treated groups (males/females) generally followed a dose related trend with respect to severity and time of onset.

Bone marrow smears were also prepared at the time of necropsy to further evaluate the hematologic changes. No dose-related changes were noted for the percentage of plasma cells, lymphocytes or eosinophilic leucocytes at any dose level for either sex. No pattern of significant dose-related changes were noted for the myeloid/erythroid ratio (M/E) in the 250 or 500 ppm groups of males or females. A significant decrease in the M/E ratio was reported in the male high dose group (1000 ppm). Females showed no statistically significant decrease at 1000 ppm, however the value was lower than control value (0.74 vs. 0.82).

It was reported that one dog of the high dose male group had a low M/E and attendant hypercellularity of the bone marrow suggesting an erythroid hyperplasia. However, it appeared to the examining hematologist that the bone marrow section may have been from some other anatomical source rather than the metaphyseal protion of the long bone as were all the other samples. The cellularity of the bone marrow in all the other dogs of the high dose group was judged, by the hematologist, to be within normal limits and not different for the control group. The decreased M/E ratio in males with no discernable change in bone marrow cellularity and no decrease in peripheral blood leucocyte count suggested that the decreased hematocrit, decreased RBC count and decreased hemoglobin concentration were accompanied by a mild erythroid hyperplasia of the bone marrow. No changes in the morphology of the bone marrow cells were detected.

Hermosiderin was evident in the marrow smears of all dogs in the high dose group except the one dog noted previously. A special stain for iron was not performed.

Clinical Chemistry: Males: Parameters showing salient changes were phosphorous, total cholesterol alkaline phosphatase and SGPT. Statistically significant decreases in phosphorous levels were recorded for the mid (500 ppm) and high (1000 ppm) dose groups at 1, 2, 3, and 4 months and 1, 2, 3, 4, 5 and 6 months respectively. Statistically significant increases were observed for total cholesterol in the low dose (250 ppm) at 1, 3, 5 and 6 months; for the mid dose (500 ppm) at 1, 4, 5 and 6 months and the high dose (1000 ppm) at 1, 2, 3, 4, 5 and 6 months. Values also showed an increased elevation with increased dose at comparable time intervals. Alkaline phosphatase levels for controls and the low dose group were comparable with generally decreasing values with increasing time. Values for the mid and high dose groups appeared dose responsive with the mid and high doses being statistically significantly increased over control values. SGPT values were also statistically significantly increased for the high dose group at 1, 3, 4, and 5 months. Statistically significant values were also

recorded for other parameters, however, neither values nor statistical significance was consistent with dose or time for the parameters where the changes occurred.

Females: Parameters showing salient changes were alkaline phosphatase and total cholesterol. Alkaline phosphatase levels were not statistically significantly different from control values for the low dose group (250 ppm). Values at the mid dose were not statistically significantly different from control values and trended irregularly downward with time as expected. Alkaline phosphatase levels in the high dose group were statistically significantly elevated above control values at 3, 4, 5 and 6 months. Total cholesterol values were statistically significantly higher only at months 1 and 2 of the high dose group. Statistical significance in other parameters was neither consistent nor log-dose responsive.

Urinalysis: Values for treated and control groups were generally comparable. Urobilinogen values were also comparable for all groups.

Ophthalmologic Examination: No abnormalities which would affect the study were observed in any of the dogs subsequently assigned to the treatment groups. A number of dogs from all groups exhibited minimal to mild bilateral conjunctivitis prior to being placed on test. The inflammatory changes were considered to be related to the particulate debris from the powdered diet. It was reported that the particulate debris tends to occlude the lacrimal drainage system, and predisposing the eye to stagnation and normal tear flow and ultimate inflammatory change (Note: These changes were also evident in several animals at the six month examination).

Bilateral mild to moderate increase in retinal tortuosity was observed at the following dose levels:

Dose (ppm)	Sex	No. Obs.	Sex	No. Obs.
0	M	0	F	0
250	M	0	F	0
500	M	2	F	2
1000	M	4	F	2

It was noted that although a certain degree of variation in vascular tortuosity was not uncommon in the beagle, the changes observed were considered to be greater than the normal limits. The ophthamologist concluded that the findings were compound related since lesions were seen in the mid and high dose animals only.

Neurological Examination: The neurologist noted that most dogs had irritated, reddened and swollen skin between the pads of the feet. This lesion was observed in all groups of dogs and it was suggested by the neurologist that cage management or cage design was responsible for the lesions. However, also noted was the fact that the yellow (500 ppm) and red groups (1000 ppm) had a more severe irriation and that many of them also chewed their feet (biting of the extremities).

It was also reported by the neurologist that many of the dogs appeared to have a stoical attitude to tacticle stimulation around and on the eyes and that this diminished or absence of response was present in all dose groups. It was noted by the neurologist that the food that the dogs ate was produced in a fine powder and when the dogs ate, the powder was blown around their cage and most dogs had food stuck to their muzzle and mouth up to 3 hours after eating. This led the neurologist to the opinion that because of the powdered food and the prolonged dusty cages there may have been a continued ocular irritation producing an adaption to tacticle stimulation to the eye. These findings also correlate to some degree with the result of the ophthalmological examination.

White Group (control group): It was reported that some of the white group were evaluated each evaluating period at which time they appeared bright, alert, and responsive and exhibited no gait abnormality. A few of the dogs did not want to walk on the unfamilar floor. No other specific abnormalities were detected with any degree of consistency.

Blue Group (250 ppm): It was reported that no deficit could be detected in mental status or posture and gait. It was noted that spinal reflexes were variable, as expected, and mildly depressed to normal. Three dogs were found to have less than expected control of their muzzle facial muscle. This was considered a neurological deficit in cranial nerve function. (Each dog's muzzle control was tested by pinching the upper lip and observing the response. The response requires cerebrocortical control and is mediated via the facial nerve.) Furthermore, 2 of the 3 dogs had been examined previously and were determined to have mild postural deficits as well.

Yellow group (500 ppm): One dog showed mental disturbance. (see also paragraph on mortality). The neurologist reported that when the animal was examined prior to a day's rations and approximately 3 hours after that day's rations, there was a disturbance in response to surrounding activity and noise with the animal overreacting to the stimuli.

A change in gait was also observed in this animal. It was also reported that this dog's head swung on wider excursions (DO 286) as he moved. This sign was noted during the post-prandial observation period and was suggestive of bilateral vestibular disturbance.

A second dog (DO 287) of this group exhibited a change in gait between pre- and postprandial times. A normal gait was detected preprandial and 3 hours after feeding the dog exhibited a broad based stance, trunkal ataxia and an exaggerated head movement (intention tremor). The neurologist noted that all 3 of these signs were indicative of a disturbance in the vistibulo-cerebellar function. This dog also acted slightly confused postprandial, and exhibited worse postural reactions during the postprandial observation period.

Several dogs in the yellow dose group (500 ppm) had mild to moderate disturbances in their postural reaction test. Cranial nerve reflexes were however normal.

Five dogs were found to have weak facial musculature when tested for facial muscle function about the muzzle. One dog had normal facial function during 2 preprandial exams and reduced facial function during 1 post prandial exam. Spinal reflexes were reported to be variable with a slightly more reduction in reflex activity noted.

Two dogs were chewing their feet and these same dogs were noted to have an altered response to pin pricks in the affected feet. One dog responded in an exaggerated manner and the other in a depressed manner.

Red group (1000 ppm): This group exhibited relatively normal (see also paragraph on mortality) mental status. One dog appeared to be hyper-responsive to external stimuli. The neurologist's overall opinion of the group was that they seemed less interested in their surroundings and seemed to have less curiosity of the new environment. This high dose group of dogs exhibited moderate to severe ataxia associated with an intention tremor, trunkal ataxia, and dysmecria of the limbs. This finding suggested vestibulocerebellar malfunction. Postural reactions were poor to absent in all dogs examined. Cranial nerve reflexes were relatively normal. Spinal reflexes were reduced to normal corresponding to the degree of postural deficits. Facial muscle function was considerably poorer in this group. Five dogs had absent function of facial muscle over the muzzle and 2 others were weak. It was noted that several dogs in the high dose group exhibited definite sensory deficits in the legs, feet and face. Reflex activity was not reduced to the same degree. Some dogs in the high dose group exhibited severe carpal hyperextension suggesting ulnar and/or median nerve dys-

function. It was noted by the neurologist that this particular abnormality may have been a conformational (anatomical) defect, but this observation was not noted in other groups.

Neurological summary: Mental status, gait, and posture were not severely affected except in the high dose group (1000 ppm). Transient effects were noted in the yellow (500 ppm) group. Postural deficit were also reported for the low dose group. Spinal reflexes and general cranial nerve tests were not indicative of disease.

The motor ability of the facial muscles of the muzzle appeared to be the best indicator of an adverse effect. It was explained that as this nerve or muscle is affected the muscle contracts causing a "tightness" and drawn" appearance to the dogs' faces. This facial appearance was more evident in the 500 and 1000 ppm group but did exhibit itself in the low dose group. Facial tone was found to be weak in 3 blue group dogs.

Specific sensory deficits were obvious in the high dose group and subtle in the mid dose group. The control and low dose groups appeared to have normal sensory functions.

Neurologist's Conclusion - Peripheral Nerves: There was both motor and sensory nerve involvement, with the motor involvement appearing earlier in the disease process. The reflex and sensory findings in the dogs suggest a disease process of the distal axons of the peripheral nerves. There appears to be more dysfunction in nerve fiber functions that are not associated with a heavy myelin sheet.

Neurologist's Conclusion - CNS: Definite involvement of the CNS was observed for the high and mid-dose groups. The low dose and control groups did not appear to manifest CNS involvement. In the mid dose group CNS signs appeared transient and variable and probably related to the dose interval. No cumulative effects were noted. In the high dose group (1000 ppm) CNS signs were not transient, and appeared to persist throughout the dose interval. It was speculated that the dose interval was either too short to clear the toxic compound or that cummulative effects were being manifested.

The neurologist concluded that the disease in its chronic form appeared to be a distal peripheral neuropathy not involving myelin. In its acute or transient form there appeared to be a major CNS component specifically of the cerebullar and vestibular function.

Organ Weights: Males: Changes in organ weight ratios and and absolute organ weights revealed no biologically meaningful changes for males. Females: Organ to body weight ratios

were statistically significantly increased for all treated groups. However, these effects are considered to be secondary to body weight decreases which appeared dose responsive. Absolute organ weights were not statistically significant from control values.

Gross pathology: Macroscopic findings revealed no dose related changes.

Histopathology: Hepatic microgranulomatosis was observed in 1 male and 2 female dogs of the control group, and consisted of a few small granulomas. Microgranulomas were randomly scattered through all liver lobes of treated male dogs as follows: 250 ppm dose group 2/6; 500 ppm dose group 6/6 and the 1000 ppm dose group 6/6. Microgranulomas of the liver were noted in treated females as follows: low dose 5/6, mid-dose 5/6 high dose 6/6. The hepatic lesions showed a dose response relationship with respect to incidence and severity.

Histiocytic cell infiltrate (macrophages) was present in the male mesenteric lymph nodes of 4 dogs of the high dose group and 2 dogs each of the mid and high dose female group. Histiocytic cell infiltrate was not observed in the lower dose groups of the respective sexes or the control groups.

Axonal dystrophy, characterized by swelling of the axis cylinders was present in the spinal cords of 2 male animals from the low dose group and 1 male in each of the mid and high dose groups. Similar observations were reported for 1 female of the low dose group. The control groups of either sex did not show axonal dystrophy in the tissues viewed.

Peripheral axonal dystrophy, in males, was only noted in 1 animal of the low dose group and involved the right plantar nerve. Peripheral axonal dystrophy in females was noted in the right plamtar nerve of 1 female control animal, the left ulnar nerve of 1 female of the mid dose group and the left tibial nerve of 1 high dose female.

A treatment pattern was not noted by the histopathologist. However, the micropathologist noted that it appeared that the lesions were segmental, and that random sectioning may not have revealed the true incidence of axonal dystrophy.

Mammary gland activation was also noted in 1 low dose and 3 mid dose females. No activation was noted in controls or the high dose group. Mammary gland activation was not observed in males.

A variety of other lesions were present but were not considered to be compound related.

We also note that the sciatic and facial nerves were not listed in the summary incidence table. However, inspection of the individual data sheets did reveal negative findings for all dogs for these 2 nerves.

Discussion: Food consumption was not significantly different for males or females when treated groups were compared to their respective control groups. Body weights for males in treated groups were not statistically significantly decreased when compared to the group control values. Body weight decreases for females were progressive at all dose levels and culminated in a statistically significant decrease in the high dose group. It can be stated therefore that body weight decreases in females are the result of compound toxicity and not diminished food intake. Thinness as reflected in the pharmacotoxic signs for females appeared to generally reflect the data for body weight loss. The number of males showing thinness was generally comparable for all groups, and also appeared to reflect the constancy of body weight. Biting of the extremities was noted in the control and treated groups of both sexes. Although the number of treated females for all dose groups was comparable to the control group the cumulative incidence in females, as in males, was greatly increased above values reported for the control groups. Additionally, the median response time for both sexes, but particularly for males, decreased with increased dose and appeared to be dose responsive. Based upon the above observations it was concluded that biting of the extremities was treatment related beginning with the lowest dose administered It is also pointed out here that during the neurological examination the general observations were made that of the dogs examined most dogs had irritated, reddened and swollen skin between the pads of their feet with the mid and high-dose groups of both sexes manifesting a more severe foot irritation. It was also noted that many of the dogs in the mid and high dose group also chewed their feet. A related and perhaps pertinent observation was that the dog food the animals ate was presented in dry form (fine powder) and as the dogs ate the powder was blown around the cage and dusted the cages for prolonged periods of time. The neurologist further elaborated by stating that most dogs had food stuck to their muzzle and mouth even 3 hours after eating and that the food "dust" particles may well have been responsible for a continued ocular irritation. In light of the information presented one might conclude that the biting of the legs, paws, and tail may have been to some extent a secondary effect caused by the topical contact and resulting irritation of the treated diet. However, this position is mitigated somewhat by the findings of the neurological exam to be noted later in this review, as well as the available histopathology. The central nervous system (CNS) mediated pharmacotoxic signs of emesis

and head shaking were noted in males at the low dose and occurred earlier in time and dose administered than ataxia and tremors. The ataxia and tremors noted in the mid and high dose male groups was considered treatment related increasing in both frequency and number of animals affected. Ataxia and tremors were not observed at the low dose or in the control group. Head shaking in males was not observed in the control group but showed a progressive increase in the number of animals affected and cumulative incidence with dose. The decrease in onset time was dose responsive. Emesis was also compound related as indicated by a decreased time to onset which occurred in a dose response manner. It is also noted here that histopathological examination of the digestive tract gave little indication of compound irritation.

Emesis, head shaking, ataxia and tremors are considered by this reviewer to be progressive and dose related effects on the nervous system of male dogs beginning with the lowest dose administered.

The reasoning applied to males is also applicable to females, even though the pharmacotoxic signs reported for females are not as definitive in the respect to time and dose as they are for males.

Specific neurological exams led to a conclusion by the examining neurologist of definite involvement of the CNS of the mid and high dose groups, whereas the low dose and control groups appeared to be spared from CNS involement. It is pointed out here however that 2 dogs of the low dose group were determined to have mild postural dificits. Based upon what was observed at higher doses we believe these signs to be compound related CNS effects. The observations of ataxia, intention tremors, and dysmetria of the limbs appear supportive of the pharmacotoxic signs of head shaking, ataxia and tremors. Neurological signs were reported as transient (temporary) and no cumulative effects were noted in the mid dose group, however for the high dose group signs were not transient but continuous in the opinion of the examining neurologist.

The neurologist also concluded that a progressive disease of the peripheral nervous system (legs, feet and face) was present involving both motor and sensory nerves. The reflex and sensory findings indicated a disease process of the distal axons of the peripheral nerves. The neurologist was of the opinion that the dysfunction in nerve fiber functions was associated primarily with those fibers not associated with a heavy myelin sheath. It was also apparent that the neurologist attributed the reduced muzzle facial muscle function (low, mid, high dose) to a peripheral neuropathy rather than to the influence of a topical irritation (or effect)

brought about by the formulated diet mentioned earlier. Histopathology of the nervous system did not appear to be supportive of the neurological findings or pharmacotoxic observation as a definitive treatment pattern of histological findings not observed. However a caveat was issued in that the histopathological lesions which were observed appeared to be segmented (localized) and that random sectioning may not have revealed the true incidence of axonal dystrophy in test animals.

Histopathology of the liver indicated that hepatic micro-granulomatosis was present in males and females of all groups. However hepatic lesions showed a dose response relationship with respect to both incidence and severity and were considered to be treatment related. This effect in liver may to some degree explain the increased serum cholesterol, alkaline phosphatase and SGPT levels.

Decreased erythroid parameters in the blood and the erythroid hyperplasia of the bone narrow could be related to mild blood loss, hemolysis or secondary changes in other tissues. The condition appeared to be one of a normocytic anemia in both sexes but predominantly in males. The cause of the anemia is not readily evident based on the data submitted.

Organ to body weight ratios were not biologically meaningful and generally reflected body weight changes.

Conclusion: Review of the petitioner's study has led to the conclusion that a no-observable-effect-level has not been established in this six-month dog study. The lowest effect level (250 ppm) has resulted in the following effects:

Emesis
Headshaking
Biting of the extremities
Normocytic anemia
Increased serum cholesterol levels
Possible CNS and peripheral nerve dysfunction
Hepatic microgranulomatosis

Core Classification: Guideline

Recommendation: It is recommended that this study be re-conducted at three (3) Appropriate dose levels.

It is also suggested (only suggested) that this compound be given intravneously to one or two dogs (of any sex, type or breed) and the animal observed only for immediate effects. This study would be considered as a supplementary study but may provide useful information. This same consideration could also be applied to the photodegradate SD-54597.

History:

ENCLOSED WITH [illegible]

FROM [illegible] TO [illegible]

Date 2603 30404

2/2 Pg

Mental Status: -

General Condition: normal

Gait & Posture:

TOX LAB
WESTHOLLOW RESEARCH CENTER
RECD FEB 20 1981

1-cmr

Charges _____

Cranial Nerves:

Observation:
Head
Eyes
Ears
Mouth

Reflexes:
Menace (II)
Pupillary (II,III)
Palpebral (V,VII)
Corneal (V,VI)
Gag (IX,X)

normal

Vestibular (VIII) Nystagmus
Resting
Positional
Vestibular
Postrotatory

normal

Spinal Reflexes: 0 to +4 (+2 = Normal)

REAR	L	R
Patellar		
Gastroc		
Cr. Tribial		
Flexor		
Crossed Ext		
Perineal (±)		

FRONT	L	R
Biceps		
Triceps		
Flexor		
Crossed Ext		
Panniculus (±)		

Postural Reactions:
Wheelbarrow
Hopping
Ext. Post. Thrust
Hemistand/walk
Placing:optic
tactile
Proprioceptive positioning

good

Sensory Testing:

good sensation throughout

Conclusion:

Best of White

Other Tests:

Neurologic Exam

White RD WTF

History:

ENCLOSED WITH [illegible] FROM [illegible] TO [illegible]

Date

26 [illegible] 1041

2/2 PM

Mental Status: normal

General Condition:

Gait & Posture:

TOX LAB
WESTHOLLOW RESEARCH CENTER
RECD FEB 20 1981
1-cmr

Charges

Cranial Nerves:

Observation:
Head
Eyes
Ears
Mouth

Reflexes:
Menace (II)
Pupillary (II,III)
Palpebral (V,VII)
Corneal (V,VI)
Gag (IX,X)

normal

Vestibular (VIII) Nystagmus
Resting
Positional
Vestibular
Postrotatory

normal

Spinal Reflexes: 0 to +4 (+2 = Normal)

REAR	L	R
Patellar		
Gastroc		0
Cr. Tibial		1
Flexor		
Crossed Ext	1	
Perineal (±)		

FRONT	L	R
Biceps		2
Triceps		
Flexor	X	
Crossed Ext		
Panniculus (±)		

Postural Reactions:
Wheelbarrow
Hopping
Ext. Post. Thrust
Hemistand/walk
Placing:optic
tactile
Proprioceptive positioning

good

Sensory Testing:

good sensory perception

Conclusion:

Best of White

Other Tests:

28

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LEVEL

00404

PROJECT 208-B SUMMARY OF TREATMENT RELATED PHARMACOTOXIC SIGNS WITH CUMULATIVE INCIDENCES[a] AND MEDIAN RESPONSE TIMES[b]

SIX-MONTH DIETARY FEEDING STUDY IN DOGS

PHARMACOTOXIC SIGN	GROUP NO.	MALES 0	MALES 250	MALES 500	MALES 1000	FEMALES 0	FEMALES 250	FEMALES 500	FEMALES 1000
		1	3	5	7	2	4	6	8
BITING EXTREMITIES									
Number of animals affected		1	5	6	6	5	6	6	6
Cumulative incidence		2	52	113	97	15	100	71	166
Median response time		157	122	16.5	6.5	50	79	15.5	15
HEAD SHAKING									
Number of animals affected		0	2	3	6	4	3	5	6
Cumulative incidence		-	6	6	58	8	3	12	140
Median response time		-	160	23	8	160	185	104	66.5
ATAXIA									
Number of animals affected		0	0	5	6	0	0	6	6
Cumulative incidence		-	-	21	268	-	-	23	425
Median response time		-	-	8	4	-	-	6.5	1.5
TREMORS									
Number of animals affected		0	0	4	6	1	1	2	6
Cumulative incidence		-	-	7	23	2	1	2	70
Median response time (days)		-	-	7	15	10	120	95.5	7
THIN									
Number of animals affected		1	1	2	0	0	3	1	5
Cumulative incidence		19	1	100	-	-	64	5	454
Median response time		180	194	54.5	-	-	33	44	44
EMESIS									
Number of animals affected		5	5	5	6	4	6	5	6
Cumulative incidence		12	8	13	20	13	15	34	23
Median response time		107	31	3	1	23	6.5	5	1.5

[a] Cumulative incidence is given as the total number of times the sign was observed in each group throughout the treatment period.

[b] Response time is given as the median time (in days) from study initiation to the first observation of the sign for each group.

Please Read over the histopathology and let me know what you think. Louis

Albino

Parameter	Group	Sex		Statistical variation
Brain weight	1	♂	MALE	S+
Adrenals Ratio	3	♂		S+
Liver Ratio	5	♂		S+
Heart Ratio	4	♂		S+
Adrenals Ratio	4	♀	FEMALE	S+
Adrenals Ratio	6	♀		S+
Adrenals Ratio	8	♀	see body wts.	S+
Liver Ratio	4	♀		S+
Liver Ratio	6	♀		S+
Liver Ratio	8	♀		S+
Ovaries Ratio	4	♀		S+
Ovaries Ratio	6	♀		S+
Ovaries Ratio	8	♀		S+
Kidneys Ratio	4	♀		S+
Kidneys Ratio	6	♀		S+
Kidneys Ratio	8	♀		S+

The sporadic differences noted for the male treated groups were considered to be physiologically insignificant and attributable to the variation normally seen in male Beagle dogs of this age. The increased mean organ/body weight ratios for the adrenals, liver, ovaries, and kidneys of all female treated groups were considered to be secondary to the lower body weights noted for these groups, rather than directly related to compound administration, as no absolute increases in the weights of these organs were apparent.

Histopathology

Detailed histopathology for each animal is presented in Appendix II, and an individual summary of these evaluations is presented in Appendices 10A and 10B. Microscopic pathologic lesions are summarized by group in Table 10.

Hepatic microgranulomatosis was observed in treated and control dogs. For the control dogs, this finding consisted of a few small granulomas and was present in only one male and two females. In the treated dogs, the granulomas were randomly scattered through all lobes in the livers of Group 3 males (2 of 6), Group 4 females (5 of 6), Group 5 males (6 of 6), Group 6 females (5 of 6), Group 7 males (5 of 5), and Group 8 females (6 of 6). The lesions were generally present within the sinusoids and were comprised of

cells resembling epithelioid cells. An occasional granuloma was encircled by a single layer of small round cells with extremely dark nuclei. The cytoplasm of the epithelioid cells resembled that of hepatocytes, and occasionally contained one or more dark brown granules resembling hemosiderin. The hepatic lesions showed a dose relationship with respect to incidence and severity, and are considered to be treatment related.

Elsewhere in the liver, foci of mononuclear cells, some resembling hematopoietic elements, were occasionally present. Hepatocytes caught up in these aggregates appeared to be undergoing necrosis.

Histiocytic cell infiltrate was present in the mesenteric lymph nodes of dogs from Groups 6, 7, and 8 (incidences of 2, 4, and 2, respectively). These cells were large with fine, light, granular, eosinophilic cytoplasm with eccentric nuclei. An occasional multinucleated form was also observed. These cells were present in the subcapsular sinuses contiguous to the cortical nodules, and were usually multifocal. The nature and source of these cells resembling histiocytes is not clear.

Axonal dystrophy, characterized by the swelling of axis cylinders, was present in the spinal cords of single animals from Groups 4, 5, and 7, and two animals from Group 3. Peripheral nerve axonal dystrophy was scattered through most groups, including the controls, and usually involved a single animal. The lesions were found in the sciatic, tibial, ulnar, and plantar nerves. A treatment related pattern could not be ascertained; however, it appeared that these lesions were segmental, and the random sectioning may not have revealed the true incidence of axonal dystrophy in these animals.

Mammary gland activation, characterized by acinar proliferation and intraductal accumulations of eosinophilic fluid, occurred only in the low and mid dose females (Groups 4 and 6). This finding was not observed in the high dose (Group 8) females, but the possibility of a treatment related effect in the mammary glands of the Group 4 and 6 dogs cannot be ruled out.

A variety of other lesions were present in both treated and control dogs. These were interpreted as spontaneous and not related to the administration of the test material.

File last updated 3/17/83

004041

ACCEPTABLE DAILY INTAKE DATA

Rat, Older NOEL mg/kg - ppm		S.F.	ADI mg/kg/day	MPI mg/day(60kg)
12.500	250.00	100	0.1250	7.5000

Published Tolerances

CROP	Tolerance	Food Factor	mg/day(1.5kg)
Cottonseed (oil)(41)	0.200	0.15	0.00045
Peanuts(115)	0.020	0.36	0.00011
Potatoes(127)	0.020	5. 3	0.00163
Soybeans (oil)(148)	0.050	0.92	0.00069
Cabbage,sauerkraut(22)	10.000	0.74	0.11037
Pecans(118)	0.200	0.03	0. 0009
Cantaloupe(23)	1.000	0.52	0.00782
Honeydewmelons(71)	1.000	0.03	0.00045
Pumpkin,inc squash(131)	1. 00	0.11	0.00169
Watermelon(169)	1.000	1.43	0.02146
Muskmelons(98)	1.000	0.03	0.00045
Pears(116)	2.000	0.26	0.00766
Apples(2)	2.000	2.53	0.07590
Cucumbers,inc pickl(40)	0.500	0.73	0.00544
Tomatoes(163)	1.000	2.87	0.04312
Summer squash(155)	0.500	0.03	0.00023
Milk&Dairy products(93)	0.100	28.62	0.04292
Cattle(26)	1.000	7.18	0.10777
Goats(62)	1.000	0.03	0.00045
Hogs(69)	1.000	3.43	0.05151
Horses(208)	1.000	0.03	0.00045
Sheep(145)	1.000	0.19	0.00291
Cauliflower(27)	0.500	0.07	0.00054
Broccoli(19)	2.000	0.10	0.00307
Beans,dry edible(10)	0.250	0.31	0.00116
Corn,grain(66)	0.020	1.00	0.00030
Peas(117)	0.250	0.69	0.00261
Peaches(114)	10.000	0.90	0.13490

MPI	TMRC	% ADI
7.5000 mg/day(60kg)	0.6261 mg/day(1.5kg)	8.35

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Unpublished,Tox Approved #1725,2143,2489,2599,2626,1E2493,2648,265

CROP	Tolerance	Food Factor	mg/day(1.5kg)
Poultry(125)	0.400	2.94	0.01766
Eggs(54)	0.150	2.77	0.00631
Celery(29)	5.000	0.29	0.01[illegible]86
Lettuce(84)	10.000	1.31	0.19622
Filberts(56)	0.200	0.03	0.00009
Lettuce(84)	0.000	1.31	0.00000
Sorghum(147)	5.000	0.03	0.00225
Sugar,cane&beet(154)	0.050	3.64	0.00273
Sunflower(156)	1.000	0.03	0.00045
Sugarcane (214)	2.000	0.03	0.00090

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Corn,Sweet(40)	0.050	1.43	0.00107
Cherries(30)	10.000	0.10	0.01533
Barley(6)	0.05	0.03	0.00360
Wheat(170)	8.000	10.36	1.24354
Oranges(108)	2.000	2.17	0.03580
Grapefruit(65)	2.000	0.99	0.02974
Lemons(82)	2.000	0.17	0.00521
Peas(117)	0.250	0.69	0.00201
Almonds(1)	0.200	0.03	0.00009
Eggplant(53)	1.000	0.03	0.00045
Peppers(120)	1.000	0.12	0.00134
All foods(197)	0.050	100.00	0.07500

IRI	TMRC	% ADI
.5000 mg/day(60kg)	2.3112 mg/day(1.5kg)	30.32

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004041

Subject: Life Span Chronic Toxicity Study of S5602 in Mice.

Test Compound: S5602 (fenvalerate, SD 43775) Technical. Lot. No. 71239. Purity: 91.4%. The compound was synthesized by [redacted] The test compound was stored in a refrigerator at 4 ± 2°C.

Accession No: 246563 and 246564

Testing Facility: Laboratory of Biochemistry and Toxicology, Research Department, Pesticides Division, Sumitomo Chemical Co. Ltd., Hyogo, Japan.

Study No: Document No. AT-10
Ref. No. - 0286

Testing Period: July 12, 1978 - April 13, 1981

Report Submitted to Sponsor: ca. November, 1981

Objective of Study: This study was supplementary to a previous study, "Eighteen-month chronic toxicity study of S5602 in mice" and was intended to establish a NOEL with respect to granulomatous changes in lymph nodes, spleen and liver.

Materials and Methods: Diet preparation and analysis. The diet for each test group was prepared at Nippon Formula Feed Manufacturing Co. Ltd., Chita factory, Aichi, Japan. The test compound was dissolved in an appropriate volume of corn oil, and then the solution was manually mixed with 10X its weight of pulverized basal diet (CE-2 type, Clea Japan, Inc.) using a sieve to make a premix. The premix was thoroughly mixed with pulverized basal diets using a mixer. The final dietary concentration of the test compound was 10, 30, 100 or 300 ppm. The final concentration of the corn oil in the diet was adjusted to 2%. Diet was prepared freshly each week during the week prior to its use for the first 32 weeks and at 4 week intervals thereafter. Test compound in the diet was shown to be stable at 4°C for at least 6 weeks. The prepared diets were kept in a refrigerator until used. The diet samples collected for concentration analysis were stored frozen (-10°C) until analysis.

Animal husbandry: Five mice of each sex were housed in a polycarbonate cage in which wood shavings (Betta Chips®, Northeastern Corp., U.S.A.) were used as the absorbent material. Each cage bore a color coded card listing the individual animal number, sex, cage number and dose group.

Each cage of mice was supplied with 1 week's ration for an ad libitum feeding. Food dispensers were checked daily and both feeder and diet were changed weekly. Water was supplied by an automated watering system and volume recorded. Rooms were monitored for temperature, humidity and a 12-hour light/dark cycle. Experimental animals: Four-week-old mice (320 males and females) of the ddy strain were purchased from Shizuoka Agricultural Cooperative Association for Experimental Animals. Mice were allowed a 1-week accommodation period. After the quarantine period, 250 animals of each sex were weighed and allocated to one of 5 experimental groups. Animals were placed into each group on a random basis and each group had a similar mean starting body weight. Feeding of the test compound was initiated when the animals were 5 weeks old and continued for 20 months, or until the mortality of the groups approached or reached 70%.

Experimental design was as follows:

Group	Diet Level (ppm)	Number of Animals	
		Males	Females
Control	0	50	50
T-1	10	50	50
T-2	30	50	50
T-3	100	50	50
T-4	300	50	50

Observations and Examinations. Clinical observations were made twice daily. Palpatation was conducted monthly after 11 months of feeding. Dead animals and those sacrificed in a moribund state were necropsied and subjected to a histopathological examination. Body weights were determined weekly for the first 3 months, twice a week for months 3-6 and every 4 weeks thereafter.

Food and water consumption were recorded on a cage basis (note: 5 animals per cage per sex) for 3 consecutive days. Food consumption was recorded weekly. Water intake was recorded following the same schedule as for body weights. Ophthalmological examinations were carried out at terminal sacrifice on the cornea, iris, lens and retina of all surviving animals using an ophthalmoscope. Eyes were dilated prior to examination with ophthalmic eye drops. Urinalysis was conducted on 10 male and 10 female mice from each gorup during the last week of feeding. Parameters measured were:

pH
glucose
protein
occult blood
ketones
bilirubin

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All surviving mice were fasted overnight. A blood sample was taken from the ophthalmic plexus and subjected to hematological and biochemical analysis as noted below:

Hematology:

erythrocyte count (RBC)
total leukocyte count
thrombocyte count
hematocrit (HCT)
hemoglobin concentration (Hgb)
mean corpuscular volume (MCV)
differential leucocyte count

Clinical biochemistry:

total protein
albumin
glucose
blood urea nitrogen
bilirubin
cholesterol
creatinine
SGOT
SGPT
alkaline phosphatase
LDH
A/G ratio
leucine aminopeptidase

Organ weights were determined for brain, lung, heart, liver, kidneys, spleen, testes (ovaries), pituitary and adrenals.

Necropsy examinations were conducted upon all surviving animals at termination and those found dead or moribund unless precluded by severe autolysis. Representative tissues and organs including tissue masses were fixed with 10% neutral formal-saline or Bouin's fixative, embedded in paraffin wax and sliced into sections. Sections were stained with H and E and examined under a light microscope. The following organs and tissues were examined:

brain
eye
spinal cord
sciatic nerve
salivary gland
liver
gall bladder
pancreas

trachea
lung
heart
spleen
bone marrow
lymph nodes
  mandibular
  mesenteric
  others
thymus
tongue
esophagus
stomach
intestine (small & large)
mammary gland (females only)
tissue masses
gross lesions

kidney
urinary bladder
epididymis
testis
prostate
seminal vesicle
preputial gland
ovary
uterus
pituitary
thyroid
parathyroid
adrenal
skin

Statistical Analysis using the Student's t-test was conducted on the mean values of body weight, food consumption, water intake, organ weight, hematology and biochemical values. Cumulative mortality data were also statistically analyzed using contingency tables.

Results:

Clinical Observations: Signs such as alopecia, scab formation, sparse hair, sores, scars, wasting, potbelly, leaness, swelling and palpable masses were observed. The incidence of these clinical signs were not apparently dose-responsive.

Mortality: Mortality was reported as follows:

Dose	Males	(%)	Females	(%)*
0	30	(60)	34	(68)
10	30	(60)	33	(66)
30	32	(64)	32	(64)
100	29	(58)	29	(58)
300	35	(70)	29	(58)

*Females terminated at 87 weeks. Experiment ran for 91 weeks.

Additionally it is pointed out that graphical representation of the data revealed parallel death rates between treated and control groups. Females generally began dying earlier than males (40 weeks vs. 52 weeks). Males, however, showed a slightly steeper slope than females.

Body Weight Changes: Male body weight changes in the treated group were generally comparable to the control group.

Female body weight changes for treated groups were comparable to the control group, except for the period 54-66 weeks when values were statistically significantly lower. It was noted that during this period 9/29 females died.

Food consumption (g/kg/day) and water consumption were generally comparable between treated and control group. Statistically significant changes were transient and did not appear to be biologically meaningful.

Urinalysis data revealed generally comparable values for ketones, occult blood, bilirubin, glucose, protein and pH between treated groups and control group.

Hematology: Males: Statistically significant but not log dose responsive decreases were reported for RBC values at 10, 100 and 300 ppm. The value at 30 ppm was lower but not significant. HCT, Hgb and MCV values were generally not supportive of the decreased RBC values. HCT and Hgb were statistically significantly lower only at 100 ppm and MCV was statistically significantly increased at 300 ppm. Females: Values for parameters measured were generally comparable to controls. Statistically significant but not log dose responsive decreases were reported at 30 ppm for RBC and HCT and for hematocrit alone at 300 ppm. These changes appeared to be random and not mutually supportive.

White Blood Cell Count: Males and Females: In those cases where RBC values were decreased it was noted that WBC count was increased over controls but not statistically significant. The re-examination of the individual animal data revealed that the higher leucocyte counts were attributable to a leukemia which was not compound related. Exclusion of animals manifesting leukemia and re-analysis of the hematology data revealed statistically significant decreases in RBC values for males accompanied by statistically significant increases in MCV at 100 and 300 ppm and may be reflective of a trend. Females manifested a substantial decrease in RBC count of 300 ppm which was not statistically significant but which appeared to be supported by a statistically significant decrease in Hgb value.

White Blood Cell Differential Count (Lymphocytes, neutrophiles, eosinophiles, basophiles, monocytes): Values were not statistically significant from control values for any dose level.

Biochemistry: Values for males and females appeared random and not log dose responsive. Males: Statistically significant changes were only observed at the 100 ppm dose level. Alkaline phosphatase and bilirubin values were decreased and glucose was increased. Leucine aminopeptidase was also decreased. Females: Statistically significant decreases were observed at 100 and 300 ppm for glucose. SGPT and LDH were statistically significantly increased at 30 ppm. Leucine aminopeptidase was decreased at 10 ppm.

Organ Weights (Absolute and Relative to Body Weights): The pituitary gland in males revealed an increased absolute and relative weight only at 10 ppm. Females showed a decreased adrenal weight at 100 ppm but showed no significance on a relative basis.

Gross necropsy: A summary incidence table was not provided with the report, however, it was reported that changes observed were comparable between treated and control group.

Histopathology: Granulomatous changes were observed in the mandibular and mesenteric lymph nodes as well as liver and spleen. The overall NOEL for granulomatous changes appears to be 30 ppm for males and females. The incidence of granulomatous changes, as reported by percentage, substantially increased with dose at levels of 100 and 300 ppm when compared to controls. The severity of granulomatous changes did not appear to increase meaningfully with dose and ranged between 1 and 2 (1 = slight, 2 = mild). The severity and incidence for the 7-10 animals examined for each dose level at 12 months, when compared to the severity and incidence over the life span (20 months) of all animals examined generally showed that the incidence was not progressive (and in many cases appeared regressive) and that the severity generally remained unchanged at slight to mild. Males: Giant cell infiltration was observed in the spleen only at 300 ppm. Values reported for liver with respect to granuloma, giant-cell infiltration and large histiocytes filled with brown pigment were substantially raised above control values at 100 and 300 ppm.

Values reported for mesenteric lymph nodes showed substantially raised values for reticuloendothelial cell proliferation at 300 ppm, and giant cell infiltration at 100 and 300 ppm. The values reported for the mandibular lymph node for giant-cell formation, reticuloendothelial cell proliferation and large histiocyte filled with brown pigment showed values to be

raised substantially above controls at 100 and 300 ppm. Values for the mesentric lymph node at 30 ppm for giant cell infiltration were only slightly greater than control values (0 vs 4.5 with a severity rating of 1.5. However, we also point out here that giant-cell infiltration was not observed at this level for any of the other examined tissues and that the value reported was very small. The NOEL in males appeared to be 30 ppm. Females: Giant-cell infiltration was observed at 300 ppm in spleen. Liver manifested increased values above control values for granuloma at 100 ppm, and giant-cell infiltration and granulomas at 300 ppm for the life span of the animal. Mesenteric lymph node showed raised incidence values for reticuloendothelial cell proliferation and giant-cell infiltration above controls at 100 ppm. Giant cell infiltration was observed at 30 ppm. However, the reported incidence value was extremely low (2.2 vs 0 in control) and had a severity rating of 1 (slight). Giant cell infiltration and large histiocytes filled with brown pigment were observed in mandibular lymph node at values above controls at 100 ppm. The NOEL in females for granulomatous changes appears to be the same as for males at 30 ppm.

Neoplasms (excluding leukemia): No statistically significant differences or log-dose response was reported for the following.

- The number of animals with a single neoplasm, with multiple neoplasms of the same histological type or different histological types or the total of single and multiple neoplasms.
- The number of animals with benign neoplasms only or with 1 or more malignant neoplasms.
- The number of organs with benign or malignant neoplasms per animal or their combined total.

Ophthalomological examination revealed no compound related effects.

No other compound related effects were evident when tissues were examined histologically.

Conclusion: The conclusions reported herein have been copied verbatim from the petitioner's submitted report. This reviewer agrees with these conclusions, based upon an examination of the submitted data as reflected in the reviewer's written results.

The present study reveals the following:

1. There were no clinical signs associated with S5602 administration, and mortality was not affected by S5602 throughout the feeding period.

2. Body weight change, body weight gain, food consumption and water intake were not changed by the ingestion of S5602.

3. Ophthalmological examination and urinalysis revealed no adverse effects of S5602.

4. Slight reductions of erythrocyte counts in 100 and 300 ppm groups of male and slightly lower hemoglobin concentration in 300 ppm female group were obtained.

   (Note: We also add here that the MCV values in males at 100 and 300 ppm were statistically significantly increased.)

5. Glutamic pyruvic transaminase activity of 300 ppm female group was slightly increased.

6. No noteworthy changes were obtained by ingestion of S5602 in organ weight.

7. Gross observation did not show any adverse findings related to S5602 ingestion.

8. Significantly higher incidence of granulomatous changes was observed in spleen (300 ppm group of both sexes), lymph nodes (100 and 300 ppm groups of both sexes) and liver (100 and 300 ppm groups of both sexes) by histopathological examinations.

9. S5602 was <u>not carcinogenic in ddy mouse</u> when fed from 5 weeks old to about end of life span (for 20 months).

The NOEL for this study is determined to be 30 ppm (equivalent to 3.48 and 4.29 mg/kg body weight/day, males and females respectively) based upon:

- The (slight but) statistically significant decrease in RBC count accompanied by a statistically significent increase in mean corpuscular volume (MCV) in males at 100 and 300 ppm.

- The presence of granulomatous changes in males and females at 100 ppm and 300 ppm for liver, spleen and lymph nodes (mandibular and mesenteric).

Classification: This study is classified as core-guideline for the purpose of classification in meeting the experimental objective.

Addendum: The following is added as an addendum and is a verbatim extract from the petition. The addendum compares granulomatous changes observed in this study and those observed in a previous study. The reference for the previous study is T. Suzuki, Y. Okuno, T. Hiromori, S. Ito, T. Kadota and J. Miyamoto, Eighteen-month chronic toxicity study of S5602 in mice, Technical report of Sumitomo Chemical Co., Ltd., (AT-70-0176, 1977).

The verbatim extract is as follows:

> These changes of 100 ppm and 300 ppm groups were comparable to the findings which were in 100 ppm and 300 ppm groups of previous 18-months chronic toxicity study. The granulomatous changes which were noticed in the 12 month interim study were comparable in severity to the findings of these life span feeding groups, but the incidence was higher in 100 ppm and 300 ppm groups than those in the latter group. Therefore, it was considered that the glanulomatous change were not progressive on longer feeding of the compound. The naturally-occurring granulomatous changes found in the control group apparently increased as the mice grew older.
>
> In the previous study, large histiocytic cells filled with brown pigment were not noticed as a histopathological change because the change was often observed in control mice and the incidence of the findings was not related to the compound ingestion. In this study, the change was related to the dosages. It is sometimes difficult to distinguish large histiocyte from giant-cells in granulomatous change. In the previous study, severe giant-cell infiltration was observed in 1000 ppm and 3000 ppm groups, while the finding was slight in all the groups in this study. So, the large histiocyte is considered to be easily recognized in this study.

Albin B. Kociaski

Albin B. Kociaski, Ph.D.
Toxicology Branch/HED (TS-769c)

THIS REVIEW WAS [illegible]
BY MITRE CORP. (CONTRACTOR)

004041

* CONFIDENTIAL BUSINESS INFORMATION *

MRID: N/A

* Study Type: Two Year Chronic Toxicity Study in Rats

Accession Number: ~~N/A~~ 246565, -66, -67, -68.

MRID Number: N/A

Sponsor: Shell Development Co. and Sumitomo Chemical Co., Ltd. STUDY NO. Document Code AT-1 Ref. No. -0278

Contracting Lab: Laboratory Animal Center, Nihon Dobutsu Co., Osaka, Japan

Date: April 20, 1981

Test Material: S5602/SD43775 (=Fenvalerate and PYDRIN insecticide, all designations for the synthetic pyrethroid, cyano (3-phenoxyphenyl) methyl 4-chloro-alpha-(1-methylethyl) benzene acetate). Purity not designated. (Purity is 93.4%)

Protocol:

The following description of the materials and methods used for this study was abstracted and paraphrased from the original report which was a Technical Information Record summarizing the major findings.

1. Test substance and purity: The test material was described only as technical S5602/SD43775. Purity is 93.4%
2. Species of animals: Four-week old Wistar/SLC strain rats were acclimated for one week prior to initiation of the study. The five-week old rats were divided into 5 groups of 80 males and 80 females which were housed 3 per cage for 67 weeks. Due to an outbreak of respiratory disease, all animals were subsequently housed one per cage.

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* Purpose of this review was to determine whether or not the SCL strain of Wistar Rats used in this study are or are not genetically similar to Fischer 344 Rat. See page 14 for conclusion.

3. Dosing schedule: The rats were fed diets containing 0, 50, 150, 500, and 1500 ppm S56021/SD43775 for 105 weeks (males) or 119 weeks (females). The test material was dissolved in corn oil and mixed into the diets which were given ad libitum. Diets were prepared weekly. S56021/SD43775 was stable in the diet for at least 1 week at room temperature.

4. Parameters to be examined: The animals were observed daily for physical appearance, mortality, and signs of toxicity. Individual weights were recorded weekly. Food and water consumption was measured by cage for 2 consecutive days weekly. Hematology, clinical chemistry, urinalysis, and ophthalmologic examinations were performed on all surviving animals at the termination of the study. Rats which died or were sacrificed when moribund and all rats surviving at the termination of the study were subjected to a gross pathologic examination. Organs and tissues obtained from each animal were examined microscopically.

5. Statistics used: Data was analyzed by X^2 analysis.

Results:

The following observations were summarized in the Technical Information Record. 1) Clinical chemistry, urinalysis, and ophthalmological examinations revealed no treatment-related changes.

2) Except for increased mean neutrophil counts in 1500 ppm male rats, no treatment-related changes were observed in hematologic values. 3) Slightly higher mean relative weights of brain, kidneys, thyroids, and testes were observed in the 500 and 1500 ppm groups relative to controls. Since the organ weight changes were related to lowered body weights, they were considered to be of no toxicologic significance. 4) Microgranulomatous changes were observed in the lymph nodes, adrenals, spleen, and liver of both treated and control rats, but the incidence and severity of the changes were dose related only in the 500 and 1500 ppm groups. 5) Except for testicular interstitial tumors in males, distribution of tumors (leukemia, pituitary chromophobe adenomas, pheochromocytomas, etc.) were similar in all groups. Table 1 shows that significant increases in the incidence of testicular interstitial cell tumors occurred in rats treated with 50 ppm ($p \leq 0.05$), 500 ppm ($p \leq 0.01$) or 1500 ppm ($p \leq 0.01$) S5602/SD43775 when compared with the controls.

Discussion:

Testicular tumors are not common except in Fisher 344 (F344) rats where up to 100% have interstitial (Leydig cell) tumors by 30 months of age. Unlike the conventional Wistar and Sprague-Dawley rat strains in which the incidence of spontaneous interstitial cell (Leydig) testicular tumors is absent or low (less than 5%), in the

TABLE 1

INCIDENCE OF TESTICULAR INTERSTITIAL CELL TUMORS IN WISTAR/SLC RATS FED S5602/SD43775 IN A CHRONIC STUDY

	Incidence of Tumors In Mice Fed S5602/SD43775 At:				
	0 ppm	50 ppm	150 ppm	500 ppm	1500 ppm
Final Sacrifice	6/17 35%	14/20* 70%	9/22 41%	28/29** 97%	19/22** 86%
Dead and Interim Sacrifice	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%

* $p \leq 0.05$
** $p \leq 0.01$

Subject: Species Susceptibility of Pydrin® Insecticide in the Rat, Mouse, Hamster and Rabbit. - Comments (by Shell Chemical Co. Pathologists) on Pydrin® Associated Nerve Lesions (Pathology). 004041

Accession No. 246562

Shell Project No: 61530

Printed: November 1981

Shell pathologists personally examined the original stained nerve tissue sections for histological evidence of changes in peripheral (sciatic, tibial, plantar) nerves from laboratory rats exposed to SD43775. This was done because the Shell Chemical Co. believed that the subjective grading scheme employed by the original pathologist implied a greater severity and wider distribution of damage than was present. Shell indicated that limitation of the classification scheme by the pathologist at Intermountain Laboratories to a 3 point scale (i.e., 1 = easily seen, focal or multifocal; 2 = readily seen, diffuse; 3 = extensive involvement, diffuse with inflammation) implied that a grade 2 lesion would be marked and widely distributed. Photomicrographs presented by Shell indicated that this was not the case, and that the lesions more closely fitted the definition of a grade 1 lesion, expecially in light of their focal to multifocal distribution. Additionally, irrespective of the classification question, the lesions were minimal in overall severity, poorly correlated to clinical signs and most prevelant near the cut edges of nerve sections, thus potentially confounded by artifact. Shell Co. pathologists stated that although the incidence of these lesions was correlated with exposure to Pydrin (i.e., compound related) they did not fully explain the clinical neurological signs observed in these animals.

Wistar/SLC strain of rats the spontaneous incidence of these tumors is high and variable. Table 2 shows that control Wistar/SLC rats from the same source (Shizuoka Agricultural Cooperative Association for Laboratory Animals) used in this study exhibited highly variable incidences of interstitial cell testicular tumors ranging from 11 to 98%. This high incidence of testicular tumors resembles that historically observed in F344 rats in which the incidence may reach 100%. The authors cite three studies in F344 rats, conducted by the National Cancer Institute, in which statistically significant increases in the incidence of interstitial cell testicular tumors were observed in treated rats compared to the controls. However, because of the both high and variable spontaneous incidence of these testicular tumors in F344 rats the increased incidence was not considered to be treatment-related.

The authors set out to show that the SLC strain of Wistar rats used in the study more closely resembled genetically the Fischer 344 strain rather than the conventional Wistar strains of rats by tissue typing the various rat strains and by surveying the literature for genetic markers.

Alloantisera were prepared by immunizing alloantigenic rats by skin grafts and i.p. injections of lymphoid cells. Some antisera were absorbed with erythrocytes. The dextran hemagglutination test

TABLE 2

INCIDENCES OF SPONTANEOUSLY OCCURRING TESTICULAR INTERSTITAL CELL TUMORS IN WISTAR/SLC RATS FED CONTROL DIETS FOR 24 MONTHS

Laboratory[a]	Study Initiated	Study Reported	Incidence of Tumors		
			Interim	Final	Total
A	2/1972	1974	0/7 0%	5/15 33.3%	5/22[b] 22.7%
A	12/1973	1977	0/7 0%	3/18 16.7%	3/27[c] 11.1%
B	9/1973	1977	14/20 70%	18/18 100%	32/38 84.2%
B	1974-1977	1980	-	-	74/98 75.5%
C	8/1973	1977	14/55 25.5%	43/47 91.5	57/102 55.9
D	3/1975	1978	3/5 60%	41/45 91.1	44/50 88.0%
E	1977	1980	-	-	49/50 98%

[a]A, Nihon Dobutsu; B, Institute of Environmental Toxicology; C, The Biological Research Center for the Protection of the Environment; D, Institute for Biological Science (Sumitomo Chemical Co.), E, Nihon Soda Co.
[b]Early dead animals and 4 final sacrifice animals not examined histopathologically.
[c]Early dead animals not examined histogically. Total incidence not verified by MITRE and may be 3/25 or 12%.

(Natoris' method) was used for detecting alloantigenic alleles RT1 (Aa), the major histocompatability antigen RT2, and the erythrocyte antigen or blood type.

The results, summarized in Table 3, show that the Wistar/SLC strain is similar to the F344 strain and different from the other Wistar strains and the CD strain of Sprague-Dawley rats. The Wistar/JCL strain showed considerable variation in the RT1 and RT2 haplotypes.

A literature survey of the genetic characteristics of 35 inbred and outbred strains of rats in Japan further corroborated the similarity of the Wistar/SLC and F344 strains of rat. Salient features from four strains relevant to this study are summarized in Table 4. The Wistar/JCL strain shows differences in biochemical markers from the F344 indicating considerable genetic variation in this strain.

The above observations show the genetic similarity between the Wistar/SLC and F344 strains of rat and thus suggest that the Wistar rats may possess a genetic disposition to the development of a high incidence of spontaneously occurring interstitial cell testicular tumors. Moreover, this genetic disposition to testicular tumor development is borne out by the historical evidence for a high and variable incidence of this tumor in the Wistar/SCL strain of rat used in Japan (see Table 2).

TABLE 3

RT1 AND RT2 HAPLOTYPES[a] IN SEVERAL RAT STRAINS

Alloantigenic Allele	Antiserum	Haplotype	Strain of Rat				
			F344	W/SLC	W/JCL	W/IMAMICHI	CD (SD)
RT1	K & F	ℓ	+(100%)		+(100%)		+(61.5%)[b]
RT1	K & F - ACI	ℓ		+(100%)			
RT1	K & T	ℓ & u	+(100%)	+(100%)	+(100%)	±(38.5%)	+(100%)
RT1	S & A	a	-	-	+(30.8%)	-	-
RT1	S & A	k	-	-	-	+(61.5%)	-
RT2	a	a	+(100%)	+(100%)	+(7.7%)	+(100%)	-
RT2	b	b	-	-	+(92.3%)	-	+(100%)

[a]Dextran hemagglutination test; + positive, - negative, ± weak reaction. Values in parenthesis indicate percentage of animals which gave positive results.
[b]Considered to be nonspecific since the antiserum was not absorbed with ACI erythrocytes.

TABLE 4

LITERATURE SURVEY OF GENETIC CHARACTERISTICS OF SEVERAL RAT STRAINS

Strain	Alloantigenic Systems		Biochemical Markers							
	RT1 (Aa-1)	RT2	Es-1	Es-2	Es-3	Es-4	ES-si	Cat	Amy	Hb
F344	a	a	a	a	a	b	-		a	
F344/SLC*	a	a								
Wistar/SLC*	a	a	a	a	a	b	-	a,b, b',b"	a	a
Wistar/JCL*	a,a	b,a	a,b	a	a,a' a, ab	b	-	a,b, a',b'	a,b ab	a

*Strain used for this study.

In this study an outbreak of a respiratory infection resulted in 60% mortality of male rats in the control group during weeks 64-68 (i.e., approximately 15-16 months) (see Table 5). Of the control animals dying at this time 19/48 (39.5%) had testicular hyperplasia. Deaths from respiratory infection in the other treated male groups ranged from 21.3 to 37.5%.

Interstitial cell testicular tumors typically develop in the latter stages of the life span of the rats. Table 6 shows that in Wistar/SCL rats the historical incidence of tumors was 10.0 to 16.7% at 13 to 19 months and this increased up to 100% by 20 to 25 months. The early deaths from the intercurrent respiratory infection makes it impossible to determine the true final incidence of interstitial cell testicular tumors in the control animals. From the high incidence of testicular hyperplasia in control animals dying at 64 to 68 weeks one might speculate a higher incidence of testicular tumors at termination of the study had they survived.

In studies sponsored by the National Cancer Institute regarding the bioassay of 2,5-toluendiamine sulfate, 2,3,5,6-tetrachloro-4-nitroanisole, and daminozide, in F344 rats, statistically significant increases in the incidences of interstitial cell testicular tumors were observed at several of the doses tested. Development of these tumors was not considered treatment-related by

TABLE 5

INCIDENCE OF MORTALITY FROM RESPIRATORY INFECTION
AT WEEKS 64-68 OF RATS FED S5602/SD43775 IN A CHRONIC STUDY

Response	Mortality				
	0 ppm	50 ppm	~~150~~ 150 ppm	~~500~~ 500 ppm	1500 ppm
Dead/Total	48[a]/80	30/80	24/80	17/80	24/80
Percent Dead	60	37.5	30	21.3	30

[a]Testicular hyperplasia was observed in 19/48 (39.6%) animals.

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TABLE 6

DEVELOPMENT OF INTERSTITIAL CELL TESTICULAR TUMORS IN THE WISTAR/SCL[a] RAT

Location[b]	Year Reported	Feeding Period (months)	Incidences of Interstitial Cell Tumors	
			Number Examined	Incidence (%)
A	1974[c]	13	30	10.0
		19	12	16.7
		25	68	100.0
A	1976[c]	14	50	14.0
		20	14	100.0
		26	234	98.7
B	1975[d]	24-36	91	92.3

[a]Wister rat of Shizuoka Agricultural Cooperative Association For Laboratory Animals.
[b]A, Nihon Soda Co., B, Fujisawa Pharmaceutical Co. Ltd.
[c]Intercurrent deaths not included.
[d]Deaths prior to 24 months not included.

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the expert panel since the spontaneous incidence of these benign neoplasms is both high and variable in the Fischer 344 rat. If this is considered a precedent the same rationale should hold for the Wistar/SLC strain of rat which also has a high and variable incidence of spontaneous interstitial cell testicular tumors. The evidence for genetic similiarity to the Fischer 344 rat and not to other strains of Wistar rats serves only to confirm the propensity for the high incidence of this tumor. It also suggests that the strain may not be highly inbred or that genetic drift has occurred.

According to the Technical Information Report, in addition to the intercurrent infection which resulted in 60% mortality of male control rats by week 68, the study was further flawed, namely: 1) the initial allocation of animals was not properly randomized, possibly affecting the incidence of spontaneous tumors among the groups; and 2) when the respiratory infection occurred all rats were individually housed one per cage and males previously housed separately in another room, were then interspersed among the females, thus possibly inducing hormonal changes that may have affected the incidence of interstitial cell testicular tumors.

Conclusions

Wistar/SLC rats treated in a chronic study with S5602/SD43775 at 50, 500, or 1500 ppm in the diet showed statistically significant increases in the incidence of testicular interstitial cell tumors compared to the controls. The biological significance of the increased incidence is moot because of the high and variable spontaneous incidence of these tumors in the SCL strain of rats used in Japan and obtained from one supplier. Tissue typing studies show that the SLC strain resembles the F344 strain more closely than it resembles other conventional srains of Wistar rats and Sprague-Dawley rats for which a low incidence of interstitial cell testicular tumors is common (0-5%). The similarity is corroborated by a literature survey of the genetic and biochemical markers of a number of rat strains. The genetic evidence does confirm the predisposition toward the spontaneous appearance of these tumors and suggests that the SCL strain of Wistar rats may not be highly inbred, exhibits "genetic drift" or that the strain may have been at one time bred to the Fischer 344 strain.

Core Classification: Not applicable. Information evaluated was obtained from a Technical Information Record which only summarizes major findings.

Subject: Neurotoxicity Study. The Effects of 20 Oral Doses of Fenvalerate (S 5602) Over a Period of 4 Weeks on the Rat Sciatic and Posterior Tibial Nerves and Trigeminal Ganglion.

Test Compound: Fenvalerate Technical

Accession No.: 246560

Test Facility: Shell Research Limited
Sittingbourne Research Center
London, England

Laboratory Report No.: SBER. 81.003

Testing Period: June-August 1981

Report Submitted to Sponsor: September 1981

Purity of Test Material: 93.8%.

Batch No.: 00519 (from Sumitomo Chemical Co., Osaka, Japan.

Classification: Core-Supplementary

Experimental Design: Phase 1: The first phase was designed to establish the time courses for any neuropathological or biochemical changes in the sciatic/posterior tibial nerve and trigeminal ganglion of rats resulting from the administration of 20 oral doses of fenvalerate over a 4 week period.

Phase 2: The second phase was designed to establish an oral dose of fenvalerate which when administered 5 days per week for a 4 week period would not produce neuropathological or biochemical evidence of peripheral nerve damage at the time when such degenerative changes would be expected to be maximal.

Materials/Methods

Chemicals: Fenvalerate 93.8% pure. Batch number 00519.

Dimethylsulphoxide, the solvent vehicle, was 99% pure.

Formulation: Fenvalerate was administered as a 10% (w/v) solution in DMSO (dimethylsulphoxide). Solutions were prepared every 7 days and were stable during the period of use.

Animals: Albino rats derived from the Wistar strain and bred under specified-pathogen-free conditions in the Shell Toxicology Laboratory (Tunstall) Breeding Unit were used in both phases of the experiment. Males weighed between 240-430 grams and were 11-13 weeks old. Females weighed 150-300 grams and were 11-13 weeks old.

Environmental Conditions: Animals were housed in a room with an ambient temperature of 18-22°C.

Diet: Food (LAD 1 Spillers/Spratts) and water were available ad libitum.

Phase I

Animal Allocation, Dosing Schedule, Observation and Killing Schedule.

Animal Allocation: Animals were allocated into 9 groups. Each sacrifice group consisted of 33 males and 33 females. Of these, 15 males and 15 females were designated "controls" and 18 males and 18 females were designated "fenvalerate-treated." Animals were housed 4 per cage and each animal was uniquely identified.

The statistical allocation of the animals to their sacrifice and treatment groups was made using a randomised block design. To ensure that no significant differences in body weights existed between the animals designated as fenvalerate-treated and the animals designated as control, body weights were subjected to a 2-way analysis of variance. All animals were then redistributed to individual cages.

Dosing Schedule: Control animals were administered 3 ml/kg of DMSO for days 1 and 2, and the treated animals were administered 300 mg/kg for the same period. However, from days 3-20 (with the exception of weekends when animals were not dosed) the control animals received 2 ml/kg of DMSO and the treated animals received 200 mg/kg. (This dosing schedule was chosen from a preliminary range finding study which indicated that this dosage regimen would be sufficient to produce clinical signs of intoxication in the majority of animals without causing more than 25% mortality.)

Body Weights: Body weights were determined each morning 5 days per week during the 4 week dosing period. At the end of the 4 week dosing period the frequency of body weight measurement was reduced to 3X per week. Two weeks later the frequency was reduced to 1X per week.

Monitoring of Clinical Signs: All clinical signs of intoxication were recorded every week day for the first 6 weeks of the experiment. After the 6th week all surviving animals were observed routinely on a daily basis but, unless any animal showed any signs of intoxication, formal records were only made 1X per week at the time the body weights were measured. Particular attention was paid to looking for signs characteristic of pyrethroid intoxication such as

abnormal gait and hypersensitivity to sensory stimuli such as sound. All signs of intoxication were graded for severity.

Killing Schedules: The surviving control and fenvalerate-treated rats in sacrifice groups A-I (that is 1-9) were killed 2, 3, 4, 5, 6, 8, 10, 13 and 20 weeks after the commencement of dosing, respectively. The 5 males and 5 females bearing the lowest numbers in each treatment group were sent to pathology for perfusion-fixation. The next 10 males and females from each treatment group were killed by cervical dislocation and used for the estimation of beta-glucuronidase and beta-galactosidase activity.

Phase II

Animal Allocation, Dosing Schedule, Observation and Killing Schedule.

Animal Allocation: Sixty male and 60 female rats were used. The animals were divided into blocks of 4 with each block containing animals of similar weight. The blocks were then randomised. The body weights of the 4 treatment groups were analysed by 2-way analysis of variance to ensure that no significant differences existed between the treatment group means. All animals were earmarked and individually housed in cages.

Dosing Schedule: The method, time and frequency of dosing were identical to those in Phase I. All rats received 20 doses over a 4 week period. The doses given each day to each of the 4 treatment groups were as follows:

Group W (control)	:	2 ml/kg DMSO
Group X	:	12.5 mg/kg fenvalerate (0.125 ml/kg 10% (w/v) fenvalerate in DMSO)
Group Y	:	100 mg/kg fenvalerate (1 ml/kg 10% (w/v) fenvalerate in DMSO)
Group Z	:	200 mg/kg fenvalerate (2 ml/kg 10% (w/v) fenvalerate in DMSO)

Body Weights: Body weights were measured on every weekday morning during the four (4) week dosing period. Thereafter animals were weighed 3X per week until they were killed for biochemistry or neuropathology.

Monitoring of Clinical Signs: Signs were monitored as previously noted in Phase I.

Killing Schedule: The 5 males and 5 females bearing the lowest numbers in each group were designated for neuropathological examination. The animals designated for pathology were killed 4 weeks after the commencement of dosing.

The remaining survivors from each group were killed by cervical dislocation 5 weeks after the commencement of dosing and used for the biochemical estimation of nerve damage (i.e., enzyme activity of beta-glucuronidase and beta-galactosidase). [Note: these times of killing were decided on the basis of the results from Phase 1.]

Biochemical Methods: Phase I and Phase II). The right and left sciatic/posterior tibial nerves and trigeminal ganglia were dissected free and each nerve subdivided into proximal and distal halves. Each nerve was dissected from the dorsal and ventral roots of the sciatic nerve to the distal phalangeal branches of the posterior tibial nerve. Each tissue sample was weighed and homogenized. The enzymes, beta-glucuronidase and beta-galactosidase were assayed in each homogenate using fluorometric assay techniques described in detail within the submission.

Neuropathlgical Methods: (Phase I and Phase II). Animals designated for neuropathological examination were given a lethal intraperitoneal dose of sodium pentobarbitone. Fixation (whole body perfusion) was carried out by needle injection of the solution into the ascending aorta via the left ventricle with drainage obtained via the right atrium. Animals, after fixation, were left overnight at 4°C before dissection. Samples of sciatic and posterior tibial nerve and trigeminal ganglion were removed and placed in fresh glutaraldehyde for an additional 3 hours. The samples were then post-fixed in osmium tetroxide in Dalton's chrome buffer pH 7.2 and after dehydration and treatment with propylene oxide were embedded in epoxy resin.

One micron sections of sciatic and posterior tibial nerve were cut transversely and longitudinally and stained with 1% toluidine blue. The trigeminal ganglion was divided transversely into 2 parts and longitudinal sections of each part cut and stained. The sections were examined under the light microscope and scored subjectively and independently by two neuropathologists.

Electron Microscopy: Areas from a number of animals in Phase II were selected for further examination by electron microscopy (details provided in the submission with regard to the methodology).

Statistics: Statistical procedures were limited in Phase I and were used to randomize and allocate the animals into treatment groups. A Student's "t" test was used to analyze the biochemical data.

Data from Phase II were analyzed by a number of statistical methods. Body weight data were analyzed by covariance analysis using initial body weight as the covariate. Significant differences were tested using the Williams "t" test.

Clinical signs in Phase II were compared using Wilcoxon's two-sample rank-sum test with ties. The biochemical data from Phase II was anlayzed using the Student's "t" test, the two-way analysis of variance and the Williams "t" test. Significance was taken at $p < 0.05$. Neuropathological data where appropriate were analyzed using Wilcoxon's two-sample rank-sum test with ties.

Results: Phase I: Males and females initially received 300 mg/kg/day of fenvalerate. However, after 2 days 24 males and 2 females died. The dose for both sexes was, therefore, reduced to 200 mg/kg/day for the remaining 18 days. Concurrently the dose for controls was reduced from 3.0 to 2.0 ml/kg/day. Mortality was 1% in the control group for both males and females. Mortality in the male treated and female treated groups was 20% and 9%, respectively, for the experimental duration. Signs of intoxication were observed in 98% of the treated males and 82% of the treated females. The most prevalent signs observed in the treated group were abnormal gait ("tip-toe walk, splayed hind legs and abasia") ataxia, tremor, pilerection, unkempt appearance, hypersensitivity to sensory stimuli (sound) lethargy, diarrhea, chromodacryorrhea, lacrimation and salivation. Clinical signs were observed in 33% of control males and 16% of control females. The predominant sign observed was pilerection. The data indicated a diminishing of signs in some animals receiving fenvalerate with continued dosing, however signs were not totally absent.

The sponsor also reported signs such as inflamed feet, traumatic injuries to the feet and limbs and a variety of sores and swellings for treated animals. Generally speaking, 1 female per sacrifice group showed one or more of these signs whereas 2 males (one in the 8 and one in the 10 week sacrifice group) showed any one of these signs. These signs were not observed in the control group at any time. It was therefore speculated by the sponsor that these sores and swellings which were difficult to attribute to the dosing of the pyrethroid but may have been aggravated by it. It was stated in the report that nearly all signs of intoxication disappeared within 72 hours of the last dose of fenvalerate and that no

surviving treated animal exhibited pyrethroid-related signs after the dosing period was over. Generally speaking, although the responses of individual animals differed considerably, the spectra of responses within the 9 sacrificed groups were broadly similar.

Body weight gain was decreased in those animals receiving the test compound during the dosing period. However, during the non-dosing perods (i.e., weekends) body weight gain began to recover, but again was suppressed when dosing was reinstituted.

Neuropathology was conducted on 5 animals per sex per group.

Biochemical (enzyme) determinations were conducted on not less than 8 animals per sex per group.

Enzyme Determinations: Beta-glucuronidase and beta-galactosidase enzyme levels peaked between 4 and 6 weeks after compound administration in the sciatic/posterior tibial nerves (pooled data males and females; males and females were generally affected to the same extent).

Beta-glucuronidase reached a maximum value of 150% of control value (note: control value was considered 100%, the increase above control value was therefore 50%) for both the proximal and distal portion of the nerve. Beta-galactosidase showed a maximum value of about 150% of control vaue for the distal portion of the nerve and 120% above control value for the proximal portion of the nerve.

The time course activity for both enzymes with respect to the proximal and distal portion of the nerves generally paralleled one another and did not appear to be significantly different at any time period with the exception of the 4-6 week period.

Enzyme levels for both proteins in the trigeminal ganglia showed peak values between 3 and 4 weeks ranging between 130-140% of normal. Enzyme levels for the 5th week were little changed from that observed during the preceding week. This increase was statistically significant but comparatively speaking (biologically speaking) were considered small. (NOTE: Previous studies reviewed in this branch and referenced within this report have indicated that major nerve degeneration, induced by chemicals such as mercury and acrylamide is accompanied by enzyme increases of 200% - 300% or more). Enzyme levels were within the normal range at 10 weeks for both the ganglia and nerve.

On the basis of the above data, the decision was made to kill the animals in Phase II designated for biochemical determinations during the 5th week after the commencement of dosing.

Neuropathological Determinations: Light microscopy revealed lesions resembling Wallerian degeneration in both the sciatic and posterior tibial nerves of fenvalerate-treated animals. The affected fibers showed disintegration of axons and myelin and their incorporation into vacuoles in swollen and hyperactive Schwann cells. The degenerating nerve fibres were rarely numerous and severe damage of the type following intoxication with classical neurotoxins, such as acrylamide (note: as well as mercury; see TB files Caswell No. 77A for other studies) was not observed. Lesions were scored on a scale of 1-4 (with 1 being low) by 2 neuropathologists working independently. Lesions were found in both sciatic and posterior tibial nerves and there was no apparent difference in the incidence of lesions in the proximal and distal divisions with respect to sacrifice groups. However, based upon the scoring systems used (number of animals X grade of severity = score) the highest score was attained within the first 4 weeks of compound administration. Some evidence for nerve regeneration was present in those animals sacrificed in the later stages.

Degenerating nerve fibres were found in increased numbers in treated animals from 2-6 weeks. The increases were greatest at 2-4 weeks and males appeared to suffer the greater severity. Occasionally, lesions were seen in a control animal in moderately or fairly large numbers.

On the basis of the neuropathological evidence it was decided to kill the animals in Phase II during the 4th week after the commencement of dosing (i.e., during the week immediately following the cessation of dosing when maximal changes were most likely).

Results: Phase II. Mortality was only observed in the male control group (13%) and mid-dose group (7%). Clinical signs of pyrethroid intoxication were observed in the high dose (200 mg/kg/day) group as well as the mid-dose group (100 mg/kg/day). Signs of pyrethroid intoxication appeared to be absent in the low dose groups. Body weight gains for males in the low dose group during the dosing period (Days 1-20) was comparable to controls. However, during the subsequent observation period (Days 21-35) body weights were statistically significantly lower than controls for Days 28-35. Body weight gains in the mid and high dose, for males, were statistically significantly lower at all time periods (Days 1-35). Body weight gains for females in the low dose group were comparable to controls at all time periods.

Body weight gains for females for the mid and high dose were statistically significantly lower during Days 1-25. All female groups were comparable to controls at Day 35.

Enzyme Determinations: Distal and Proximal Section of the Nerve: No increases in either enzyme were observed at the low dose administered for either sex. Males showed a statistically significant increase in beta-glucuronidase at dose levels of 100 and 200 mg/kg/day. These increases were observed in the distal and proximal section of the nerve. The percent increases ranged from 20 to 50% above control values. Betagalactosidase was statistically significantly increased only at 200 mg/kg/day. The increases were observed in both sections (proximal and distal) of the nerve and ranged from 20 to 25% above control values. Females: showed statistically significant increases of enzyme only in the distal section of the nerve. Both enzymes were elevated at 200 mg/kg while beta-galactosidase alone was raised at 100 mg/kg. Increases ranged from 20-50%. Enzyme increases were not significant in the proximal section of the nerve although increases were between 10-20% above controls.

Combined data for both sexes showed statistically significant increases at 200 mg/kg/day for both enzymes for both sections of the nerve. At 100 mg/kg/day both enzymes were statistically significantly increased in the distal section of the nerve, but not the proximal portion. At 12.5 mg/kg/day enzyme levels were comparable to control values.

Trigeminal Ganglia: Enzyme levels were not statistically significantly raised above control values at 12.5 mg/kg/day. Enzyme levels were statistically significantly increased in both sexes for the high dose. At 100 mg/kg/day betaglucuronidase was increased in males only. Data for the combined sexes showed both enzymes to be elevated at 200 mg/kg/day. Statistical significance was only noted at 200 mg/kg/day. The maximum increase was 25% above control value.

Neuropathology: Trigeminal Ganglion: No lesions were observed in any experimental group. Sciatic Nerve/Posterior Tibial Nerve: The number and severity of lesions observed in the low- and mid-dose groups were comparable to control animals for both sexes. In the high dose group both males and females showed a marked increase in the number of animals affected as well as in the severity of observed lesions for both the sciatic and posterior tibial nerve. The sciatic nerve appeared to be affected to a greater extent than the posterior tibial nerve in both sexes.

Electron Microscopy: Areas of the sciatic nerves from animals dosed at 100 mg/kg/day were selected for further examination by electron microscopy. Equivalent sections were taken from the sciatic nerves of controls. No evidence of the degeneration was found. Normal ultra-structure was observed. Additionally one animal of the high dose group showing neuropathy of the

sciatic nerve under the light microscopic was examined. Vacuolated cells containing myelin debris were clearly visible.

Discussion: Phase I of the study was conducted to (1) determine the maximum tolerated dose that could be administered to rats over a 20 day period, which would produce pharmacological/ toxicological signs but not kill more than 25% of the animals in the treated groups and, (2) to determine the optimal time period for sampling peripheral nerve tissue which would manifest a maximum response as to histopathological effects and increased levels of beta-glucuronidase and beta-galactosidase which are indicative of nerve damage.

The neuropathological and biochemical examination of the data indicated that neuropathology was most frequent and severe 2-4 weeks after the commencement of dosing, whereas associated enzyme increases were maximal between 4-6 weeks. This reviewer agrees with the sponsor that the shift in time frame does not constitute a discrepency but reflects a difference in what is being detected. Neuropathological examination would be expected to reveal all stages of nerve degeneration and in particular the early stages such as axonal breaks, whereas the biochemical method would measure enzyme changes considered to accompany the proliferation of and alteration in the functional state of macrophages, and Schwann cells which occur at a later stage (see also review by Kocialski, dated April 3, 1981. The Use of Lysosomal Enzyme Measurements as an Indicator of Chemical Induced Peripheral Neuropathy..., Caswell File No. 77A).

Neuropathology indicated that the lesions were not of the segmented demyelinating type as produced by lead or hexachlorophene, and therefore the myelin sheath is not likely to be the primary site of attack. The degeneration observed resembled Wallerian degeneration (i.e., axonal; primary effect on the axon or secondary to metabolic effects on the neurone cell body).

The neuropathological and biochemical data indicated that both the distal and proximal sections of the sciatic/posterior tibial nerve were affected. It was also noted that the proximal and distal portions of the nerve became affected at the same time. The data also indicated that both enzyme levels were consistently greater in the distal section of the nerve but it was not possible to state unequivocally whether the degeneration was of the dying back type or not, due to the relatively small differences in enzyme levels between the 2 sections of nerve. This reviewer agrees. However, in previous studies (see review by Kocialski, April 3, 1981, same review as previously referenced) enzyme levels were much higher in the distal portion of the nerve than the proximal

portion. It is noted here that neuropathological examinations were not conducted in this earlier study.

The data indicated that in animals sacrificed at 8, 10, 13 and 20 weeks the appearnace of the nerves from the fenvalerate-treated animals were virtually indistinguishable in terms of frequency and severity of lesions from those of controls. The point was therefore made that there was no evidence to suggest that new nerve damage was occurring well after dosing had ceased. This fact was also substantiated by the enzymatic data which showed that the enzyme activities declined rapidly after the peaks at 4-6 weeks. If any new degeneration was being initiated after dosing ceased the enzyme levels would have remained high.

Provided that the neurones were still viable, regeneration of nerve fibres should have occurred after cessation of dosing with fenvalerate. It should be remembered that while degeneration in a nerve fibre would be visible down its entire length the distance over which signs of regeneration can be seen may be limited. A paucity of signs of regeneration in the later kills should not be taken as an indication that it is not occurring. The fact that fenvalerate appeared to have no apparent effect on the neurones in the trigeminal ganglia and that the appearance of nerve sections of fenvalerate-treated animals at 13 weeks were indistinguishable from controls suggests that regeneration was not impaired.

There were small neuropathological indications of degenerative changes in the trigeminal roots in a small number of fenvalerate-treated animals; the incidence of these was not considered to be significantly different from the incidence in controls. However, small (i.e., 20-43%) but statistically significant increases in both enzymes were found in compound treated groups. The anatomical trigeminal ganglion taken for biochemical analysis consisted of the ganglion and its neurones and the trigeminal roots (i.e., the more proximal section of the axon of the trigeminal nerve). Increases in enzyme activities can be due either to degeneration in the roots, the ganglia neurones or both. Neurones were, however, not affected and it was thought that the enzyme changes reflected small degenerative changes in the trigeminal roots that were not visible under the microscope. It was also thought that the small enzyme increases reflected a chromatolytic response of the neurone to small degenerative changes in the trigeminal nerve.

The results of Phase II of the experiment were consistent with the results of Phase I. The animals in the top dose

dose group showed an incidence and severity of lesions and increases in beta-glucuronidase and beta-galactosidase activities comparable to the maximal effects seen in fenvalerate-treated animals in Phase I. The intermediate dose level (20 X 100 mg/kg) produced minimal signs of intoxication and a transient reduction in the rate of weight gain. Neuropathological examination at both the light and electron microscope levels revealed no significant incidence of lesions in the sciatic/posterior tibial nerves above those in controls and no abnormalities in the trigeminal ganglia. Small increases in both enzymes were, however, found. 004041

The lowest dose tested (12.5 mg/kg/day X 20 days) produced none of the neurotoxic effects of fenvalerate. There was no significant evidence for clinical signs of intoxication or any significant evidence of peripheral nerve damage (i.e. increased enzyme levels or neuropathology).

Conclusion: (1) NOEL: 12.5 mg/kg/day for 20 days (dosing period). (NOTE: Decreased body weight days 28-35).

(2) LEL: 100 mg/kg/day for 20 days. Minimal clinical signs were observed. Neuropathology was negative for sciatic and posterior tibial nerves and trigeminal ganglia. Small increases in both enzyme levels. Males appeared more affected than females.

Classification: Core - Supplementary

Subject: Species Susceptibility of Pydrin® Insecticide in the Rat, Mouse, Hamster and Rabbit.

Test Compound: Fenvalerate (SD43775) Technical

Purity: 95.8%

Accession No: 246562

Testing Facilities: Shell Development Co., Biological Sciences Research Ctr., Salida, California.
and
Intermountain Laboratories, Inc., Midvale, Utah (Pathology)

Shell Project No: 61530

Shell Study Numbers: TIR 12-002-79 (rat) 12-003-79 (mouse), 12-004-79 (hamster), 12-005-79 (rabbit)

Intermountain Project Nos: Rat (R-306), Mouse (R-371), Hamster (R-375), Rabbit (R-402).

Report Submitted to Sponsor: March 10, 1981

Study No: TIR-12-002-79 (Rat). Species Susceptibility of SD 43775.

Materials and Methods: A total of 100 male and 100 female rats (SIM:SD:BR) were obtained for the study and examined for physical, neurological and behavioral deficits. Acceptable animals were taken at random and placed into preassigned cages. All treatments, cages, and rack assignments were assigned using a computerized randomization procedure. All test animals were individually housed in an environmentally controlled room and received food and water ad libitum except as noted elsewhere in this review.

The Sprague-Dawley rats were divided into 10 groups of 10 males and 10 females per group, fasted overnight for 16 1/2-19 1/2 hours and then weighed (body weights were also taken on days 7, and 10). The animals were then placed on an inclined plane constructed of dimpled plastic for the determination of the mean slip angle. The determination of the mean slip angle was one phase of the experiment. The angle of the inclined plane was then gradually increased and the angle at which the animals began to slip down the plane was recorded (mean slip angle). The recording of these baseline values was then followed by a single acute oral dose of test material administered in a corn oil vehicle. Controls received only corn oil. A constant dose volume of 10 ml/kg

was maintained for all groups. The test doses administered were 100, 133, 180, 240, 320, 420, 560, 750 and 1000 mg/kg per respective dose group. Food was available 1 hour after dosing and water was available throughout the dosing period.

Following oral administration each animal was closely observed on an individual basis for toxic effects at 1, 2, 3, 4, 5, 6, 12 and 24 (+/-4) hours and daily thereafter until termination on day 10. The mean slip angles, as noted earlier, were recorded prior to dosing and concurrently with the observation of toxic effects at 2, 4, 6, 12 and 24 hours and daily thereafter until termination.

The significance of changes in the mean slip angles, body weight and onset and recovery times were evaluated by calculating the mean and standard error.

The AOLD50, 95% C.L. and slope of the regression curve were calculated using the log-probit method of Finney.

All surviving animals were anesthetized and perfused with saline followed by formalin. The carcasses were then eviscerated, skinned and placed in a fixative for 3 hours. Portions of the skull cap, vertebral spinous processes and skeletal muscle were then removed to expose the nervous tissue to the fixative. Each animal was then placed in an individual plastic jar and shipped to Intermountain Laboratories where additional dissection and processing was conducted. Tissue was embedded in parafin and sectioned at 5 microns. Tissue was stained with H and E. The following sections of tissue were collected:

Brain - Cross sections (3)

Spinal Cord - Cross sections (3 levels)

Sciatic Nerve - Right side; longitudinal and cross section

Tibial Nerve - Right side; longitudinal and cross section

Plantar Nerve - Right side; longitudinal and cross section. Difficulty was encountered in harvesting a viable sample from the distal plantar nerve. Therefore a section of nerve was taken that was most distal and yet represented a sample which could easily be processed.

Slides of nervous tissue containing the expected lesions were provided to Jack L. Laylor, D.V.M., Ph.D. by the Shell Toxicology Laboratory (England). Dr. Taylor, in consultation with Peter Lampert, M.D. of the Department of Pathology, University of California, San Diego, agreed that the nerve lesion provided by fenvalerate might manifest itself in a number of ways. It was therefore decided that if any one of the recognized lesions appeared it would be considered as an

effect of treatment. A classification scheme was developed which was an assessment of the severity of the lesion rather than measuring the actual manifestation of the lesion. The lesions were classified as follows:

- o axon clumping
- o myelin figures
- o vacuole formation
- o mitosis
- o inflammation

Severity of the lesion was graded as follows:

0 - no lesions observed

+/- (0.5)- barely perceptible, usually single focus

1 - easily seen, focal or multifocal

2 - readily seen, diffuse

3 - extensive involvement, diffuse with inflammation

Note: All tissues from the 4 different animal species were read blindly. The numbering system used in the pathological examination did not identify the animals as to treatment groups. Additionally, a table of random numbers was used to determine the sequence in which the animals were examined. Only after the entire study was completed were the findings rearranged into dose groups by sex.

Results: The acute oral LD_{50} determinations for males and females was as follows:

males: 938 (716-1946) mg/kg

females: 680 (569-863) mg/kg

combined: 776 (668-965) mg/kg

Animals showed good body weight gain, between all groups. Compound related effects were not observed on body weight gain.

The angle of the inclined plane was decreased with increased dose. However, an increase was found to occur with time in many of the animals including controls. The decreased angle

with dose was compound related. The increase of the mean slip angle with time within each dose group was attributed to a "learning response" since it was observed that as trials progressed with time the animals would adopt a leaning stance when placed on the inclined screen that was different from that observed with naive animals or that observed in the earlier trials (Note: the mean slip angle scores bear this point out.)

The effective dose (ED50) for toxic effects, irrespective of effects such as salivation, loose stool, diarrhea, or exopthalmus was calculated to be

males: 143 (113-168) mg/kg

females: 136 (121-161) mg/kg

combined: 141 (125-155) mg/kg

No consistent sex differences occurred in time of onset or recovery from toxic effects (Note: these data were not found in the report). The majority of animals recovered from toxic effects by 72 hours. Some animals, however, exhibited a period of apparent recovery but then became affected at a later period.

The average onset of toxic effects at lethal levels occurred between 2 hours (1000 mg/kg) and 5 hours (320 mg/kg). The most prominent effects observed included salivation, ataxia, hypoactivity, splayed fore and hind limbs, occasional head shaking, generalized course tremors that progressed in some animals to continuous convulsions, a bizarre and intermittent extension of one of both hind limbs and partial to nearly complete paresis of the limbs. Motor incoordination appeared to have the longest duration.

At less than lethal levels the toxic signs observed were more variable and less severe than higher lethal levels. The observed effects included salivation, tremors, hypoactivity, ataxia, splayed limbs, occasional head shaking and bizarre extension of the hind limbs. Partial paresis of the hind limbs was also evident in some animals as was convulsion. Motor incoordination was the most prominent sign observed and appeared to have the longest duration.

Weighted scores for sciatic, tibial, and plantar nerves were as follows:

Sciatic, Tibial, and Plantar Nerves, Weighted Scores* Incidences and Severity of Nerve Lesions - Rats.

Dose (mg/kg)	Males	Females
0	0	0
100	0	0
133	0	0
180	0.08	0.10
240	0.25	0.08
320	0.39	0.46
420	0.35	0.78
560	0.29	0.14
750	0.31	0.33
1000	0.17	1.00

*$\frac{\text{number of animals affected x severity}}{\text{number examined}}$ = score

Barely perceptible lesions were observed in the spinal cord of males at the following dose levels 180 mg/kg (1) [note: the number within the parenthesis indicates the number of animals], 420 mg/kg (1), and 560 mg/kg (1). One animal at 1000 mg/kg manifested an easily seen lesion (severity = 1) in the brain and another animal manifested a barely perceptible lesion at 750 mg/kg. Barely perceptible lesions were observed in the spinal cord of females at 420 mg/kg (2) and 750 mg/kg (1). One female at 1000 mg/kg manifested an easily seen lesion (severity = 1) of the spinal cord. Barely perceptible lesions of the brain were observed at 420 (1) and 750 (1) mg/kg. One animal at 1000 mg/kg had an easily seen lesion.

The lesions observed within the nervous tissue were treatment related. Sporadic minor lesions appeared in the low dose groups while more significant lesions and greater numbers of affected animals began to appear in the middle and high dose groups. In the high dose groups, the lesions appeared to be of greater severity, although the number of animals affected seemed to follow a "bell-shaped-curve" (i.e., normal distribution) with the highest point found somewhere in the middle dose group range. Although lesions were observed in the brain and spinal cord the greater effects were observed in the peripheral nervous system.

Except for one animal, individual rats displaying peripheral nerve lesions also showed clinical signs. Rats displaying severe clinical signs generally had more severe nerve lesions (higher scores or/and increased numbers of peripheral nerves affected). However, only 53% (i.e., about half) of the rats that displayed clinical signs of toxicity showed evidence of a nerve lesion.

The incidence by dose group is summarized below.

Dose mg/kg	100	133	180	240	320	420	560	750	1000	Combined Total
Incidence[a)]	0/0	0/8	7/16	7/18	14/18	10/15	4/14	6/12	4/5	52/98
Percent	0%	0%	44%	39%	78%	67%	29%	50%	80%	53%

a) Number of rats displaying both nerve lesions and clinical signs (> +).
Number of rats displaying clinical signs (≥ +).

A statistically significant correlation between the incidence of clinical signs of toxicity and the incidence of nerve lesions was not observed at doses around the ED50 (141 mg/kg). It was also reported that although actual numbers of rats that displayed both clinical signs of toxicity and nerve lesions tend to increase in some doses around the LD_{50}, the correlation between clinical signs and nerve lesions was not statistically significant.

Classification: Core-Supplementary.

Summary: Acute neurotoxicity studies were conducted in rats, mice, hamsters and rabbits to assess species susceptibility of peripheral nerve lesions to technical fenvalerate. Single acute oral doses ranged from a low of 32 mg/kg in mice to a high dose of 1000 mg/kg in the other 3 species. The results of the studies are contained in the individual reviews of these studies which are attached. The results generally reflect the following:

Body weights were generally not affected with the exception of hamsters which showed a decrease.

Species susceptibility as ranked by the ED50 and LD50 as follows:

Species	Combined Sex	LD50 (95% CL) mg/kg	ED50 (95%CL) mg/kg
mouse	M/F	188 (170-207)	63 (56-71)
rat	M/F	776 (668-965)	141 (125-155)
hamster	M/F	>1000.0	249 (209-294)
rabbit	M/F	>1000.0	436 (308-653)

Clinical signs of toxicity were observed with the species sensitivity following the order exhibited in the LD_{50} determinations. Prominent toxic signs observed at lethal doses were ataxia, tremors, varied fore and hind limb incoordination convulsions and death. The incidence of toxic signs were generally dose related with no obvious sex or species differences. However, in some instances, convulsions resulted in the inability of the rabbits to retract their legs from the grid floor while convulsing. The subsequent injuries sufficiently confounded the histopathology data that an unbiased interpretation of the data was stated by the sponsor to be not possible.

Histopathologic nerve lesions were tabulated 2 ways:

- o incidence of nerve lesions in individual sciatic, tibial, and plantar nerves and
- o weighted scores (incidence x severity) for combined sciatic, tibial and plantar nerves, divided by the number of nerves examined.

Nerve lesions were generally observed at doses equal to or greater than 180 mg/kg in rats and equal to or greater than 56 mg/kg in mice. Seven of 17 rats and 3 of 8 mice at lethal doses equal to or greater than 750 mg/kg and equal to or greater than 180 mg/kg of SD43775 respectively failed to show evidence of a nerve lesion. The focal nature of the lesion and individual susceptibility are probable explanations for these observations. In hamsters, nerve lesions were very sporadic without incidence or severity following any clear dose response pattern. The significance of nerve lesions in hamsters is therefore not immediately interpretable.

Weighted nerve lesion scores in rats and mice increased in a dose-related fashion (bell-shaped curve). Nerve lesions in hamsters failed to produce any consistent pathology pattern at the dose levels tested. Compound related nerve lesions were observed in rabbits; however due to mitigating factors (lesions in control animals, mechanical injury, etc.) the related effects could not be quantified.

The dose at which 50% of the rats showed a nerve lesion in a peripheral nerve is as follows:

Specie	Nerve	ED50 (mg/kg)	95% C.L.
Rat	sciatic	774.7	485-4955
	tibial	674.2	469-1735
	plantar	791.8	552-1959

Some mild lesions were also observed in the brain and spinal cord. Nine of the 10 lesions occurred in rats receiving doses >420 mg/kg. Six of the 10 rats also displayed severe clinical signs of toxicity.

Data were also examined to determine if a statistically significant correlation existed between the presence/absence of nerve lesions and the presence/absence of clinical signs. Data were examined using a 2 x 2 Chi-square test.

Rats: A statistically significant correlation between the incidence of clinical signs of toxicity and the incidence of nerve lesions was not observed at doses around the ED50 (141 mg/kg). Although actual numbers of rats that displayed both clinical signs of toxicity and nerve lesions tended to increase in some doses around the LD50, the correlation between clinical signs and nerve lesions was reported to be not statistically significant.

Mice: A statistically significant correlation between the incidence of clinical signs of toxicity and the incidence of nerve lesions was not observed in mice at doses around the ED50 (63 mg/kg) or LD50 (188 mg/kg).

Hamsters: Hamsters did not show any correlation between the incidence and severity of clinical signs and the incidence and severity of nerve lesions.

Rabbits: In rabbits, a number of suspected or observed back and leg injuries occurred during dosing. Other severe back and leg injuries occurred as a consequence of convulsions. The rabbit data was therefore not subjected to statistical analyses.

Study No: TIR-12-002-79 (Mouse). Species Susceptibility of SD 43775. (Accession No. 246562)

Materials and Methods: A total of 120 male and 120 female B6C3F1 strain mice weighing between 18 to 22 grams were obtained from Charles River Breeding Laboratories of Wilmington, Delaware. The mice were examined for physical, behavioral and neurological deficits then taken at random and placed into preassigned cages. All cages, racks, and treatment allocations were assigned using a computerized randomization procedure. All animals had free access to food and water and were housed in an environmentally controlled room. Animals were fasted overnight (16-17 1/2 hours), individually weighed, and ear punched for identification.

Nine oral dosing levels were administered to nine test groups comprising 7 animals per sex per dose. Doses of 95.8% technical administered were 32, 42, 56, 75, 100, 133, 180, 240 and 320 mg/kg. One additional group served as the control group.

The compound was administered using a constant dose volume of 10 ml/kg. Controls received only corn oil. Water was available throughout the testing period and food was returned 1 hour after dosing. Animals were observed for toxic effects at 1, 2, 4, 6, 12 and 24 hours and daily thereafter until termination on day 10. Animals were sacrificed by anesthesia and then perfused with saline followed by formalin injection. Animals were partially dissected for subsequent close dissection and histopathologic examination of neuronal tissue by Dr. J. L. Taylor of the Intermountain Laboratories (Note: procedures and scoring followed here were identical to those described for the rat study TIR-12-002-79)

The acute oral LD50, 95% C.L. and the slope of the regression curve were calculated using the log-probit method of Finney. The duration of toxic effects was also reported. The significance of changes in the lethality times, onset and recovery times, body weight and incidence of toxic signs were evaluated by calculating the mean and standard error.

Results: The acute oral LD50 was determined to be as follows:

males: 190 (164-220) mg/kg

females: 185 (160-215) mg/kg

combined: 188 (170-207) mg/kg

Prominent toxic effects observed at lethal levels (133 mg/kg and greater) included increased hypersensitivity to touch and sound stimulus generalized tremors, decreased muscle tone and death followed by rapid rigor. Convulsant activity and pronounced motor incoordinating effects were not observed at dosages less than 75 mg/kg.

The effective dose (ED50) of the principal toxic signs (combined sexes) was determined to be 63 (56-71 95% C. L.) mg/kg.

Body weight changes were comparable between treated groups and controls.

Gross necropsy of animals dying acutely revealed lung congestion, a bloated stomach with gas and/or white mucoid material and in some animals hemorrhagic areas of the mucosal surface and, less frequently a contracted heart (systole) with the presence of clotted blood. Rapid rigor was noted in a few animals. Death appeared due to respiratory failure as evidenced by cessation of respiration while a heartbeat could still be palpatated.

Microscopic examination of brain and spinal cord for males and females did not reveal the presence of lesions.

Weighted scores for sciatic, tibial and plantar nerves were as follows:

Sciatic, Tibial, and Plantar Nerves, Weighted Scores* Incidences and Severity of Nerve Lesions--Mice.

Dose (mg/kg)	Males	Females
0	0	0
32	0.02	0.02
42	0	0.02
56	0.14	0.07
75	0.14	0.02
100	0.24	0.21
133	0.07	0
180	0.33	0.67
240	1.67	0.17

*$\frac{\text{number of animals affected x severity}}{\text{number of animals affected}}$ = score

The data reflect an increased score with increased dose.

A statistically significant correlation between the incidence of clinical signs of toxicity and the incidence of nerve lesions was not observed in mice at doses around the ED 50 or LD50. Five mice showed slight histopathological lesions in the absence of any observed clinical signs of toxicity. Additionally two mice had easily to readily seen lesions but no clinical signs. This lack of correlation was noted only at the 56 mg/kg group and was distributed almost equally between sexes.

Classification: Core-Supplementary

DCR-32835:Kocialski:jad:TOX33:9/8/83
Revised:DCR-32994:Kocialski:bje:CBI-4-TOX:10/5/83

004041

Study No: TIR-12-004-79 (Hamster) Species Susceptibility of SD43775 (Accession No. 246562).

Materials and Methods: A total of 125 male and 125 female Golden Syrian hamsters (SIM:SYR strain) 5-6 weeks of age were obtained for the study. (NOTE: Only 192 were actually used). Hamsters were examined for physical, neurological and behavioral deficits. Acceptable animals were taken at random and placed into preassigned cages. Animals were housed 2 to a cage and had free access to food and water. The room temperature, humidity and lighting were controlled and monitored. Test animals were fasted overnight 16-18 hours, individually weighed, coded and administered their respective doses of 95.8% technical fenvalerate. Water was available throughout the testing period and food was returned to animals 1 hour post-dosing. The following dose levels were administered to 8 animals per sex per dose group; 56, 75, 100, 133, 180, 240, 320, 420, 560, 750, and 1000 mg/kg. of body weight. Dose volume was kept constant at 10 ml/kg. Controls received only vehicle (corn oil).

Following administration animals were observed for toxic effects at 1, 2, 4, 6, 12 and 24 hours and daily thereafter until termination on day 10.

The significance of changes in the onset and recovery times and body weight were evaluated by calculating the mean and standard error and analysis of covariance (body weight).

All surviving animals at day 10 were weighed, observed for toxic or pharmacological signs and sacrificed by I.P. injection of pentobarbital. Animals were then perfused with saline followed by formalin injection. Hamsters were then partially dissected for subsequent close dissection and histopathological examination of neuronal tissue, by Dr. J. L. Taylor of the Intermountain Laboratories. (Note: Procedures and scoring followed here were similar to that described for the rat study TIR-12-002-79).

Results: The acute oral LD_{50} was as follows:

Males: >1000 mg/kg

Females: >1000 mg/kg.

However, the death of 3/8 animals in each sex suggested that the dose of 750 to 1000 mg/kg may be a threshold level. Prominent toxic effects observed at lethal levels included hyperactivity, ataxia, generalized tremors, pronounced and varied motor incoordinating effects, intermittent clonic convulsions that progressed in some animals to continuous, clonic convulsions and death. Motor incoordinating effects were the most prominent signs observed and appeared to have the longest duration of action. No delayed effects occurred.

Body weight was statistically significantly decreased for males beginning at 180 mg/kg and for females at 420 mg/kg. However, dose related decreases did not appear evident.

The ED50 (effective dose level) for the appearance of compound related effects was calculated as follows:

Males: 286 (221-364) mg/kg

Females: 220 (166-286) mg/kg

Combined: 249 (209-294) mg/kg

Histological examination of brain and spinal cord did not reveal lesions at any dose level in either sex.

Weighted scores for sciatic, tibial, and plantar nerves were as follows:

Sciatic, Tibial and Plantar Nerves, Weighted Scores* Incidences and Severity of Nerves Lesions - Hamsters

Dose (mg/kg)	Males	Females
0	0	0
56	0.04	0
75	0.07	0.08
100	0	0
133	0	0.04
180	0	0
240	0	0.02
320	0	0
420	0.03	0
560	0.08	0.03
750	0.11	0
1000	0.02	0.03

*$\frac{\text{no. of animals affected x severity}}{\text{no. of animals affected}}$ = score

A clear pattern of lesion incidence in females was not evident. Additionally for males, at 1000 mg/kg, scores were decreased compared to previous doses. With the exception of the 750 mg/k dose where 3 animals per sex died (3/8), only 1 animal died at each dose level above 320 mg/kg. The decreased value at 1000 mg/kg was therefore not the result of an increased lethality.

It was reported that hamsters did not show any correlation between the incidence and severity of clinical signs and the incidence and severity of nerve lesions. Approximately, equal numbers of hamsters showed nerve lesions with or without clinical signs.

Classification: Core-Supplementary

Study No.: TIR-12-005-79 (Rabbit). Species Susceptibility of SD43775 (Accession No. 246562) 004041

Materials and Methods: A total of 34 male and 34 female New Zealand white rabbits (Albino Wooley Free strain) were obtained from Life Science Associates, Saratoga, California. Animals devoid of physical, neurological and behavioral deficits were taken at random and placed into their preassigned cages. All treatments, cages and rack assignments were assigned using a computerized randomization procedure.

Sixty test animals equally divided as to sex were distributed into six test groups of 5 males and 5 females per group. Animals were housed individually in stainless steel cages and had free access to food and water. The environment of the room was controlled. Following an 8 day adaptation period the animals were fasted overnight for 16-18 hours, individually weighed, uniquely marked, and administered their respective oral dose of test material. The treated groups received either 100, 180, 320, 560 or 1000 mg/kg of 95.8% technical which was administered at a constant volume of 5.0 ml/kg. Corn oil was used as the vehicle and controls recived only corn oil. Water was available throughout the testing period and food was returned to animals 1 hour after dosing.

Observations were made for signs and lethality at 1, 2, 4, 6, 8, 12, and 24 hours and daily thereafter until termination at day 10. The time of onset and recovery were recorded. The significance of changes in the onset and recovery times and body weight were evaluated by calculating the means and standard error of the mean.

Surviving rabbits were sacrificed using pentobarbital followed by exposure of the pleural cavity. Animals were then perfused with saline followed by formalin according to the procedure described for rats. The animals were then partially dissected, immersed in preservative and shipped to Intermountain Laboratories for further detailed examination.

Results: The acute oral LD50 for males and females was as follows:

Males: >1000.0 mg/kg

Females: >1000.0 mg/kg

The ED (effective [symptomatic] dose) 50 for males and females was as follows:

Males: 471 (280-954) mg/kg

Females: 398 (214-854) mg/kg

Combined: 436 (308-653) mg/kg

The prominent effects observed at the lethal dose of 1000 mg/kg included unsteady stance, generalized tremors, marked and varied fore and hind limb motor incoordination preconvulsive behavior (occasional head shaking and myoclonus) that progressed in some animals to continuous clonic convulsions and bizarre extension of one or both hindlimbs. Motor incoordination was of long duration and generally was the toxic sign upon which average recovery time was based (Note: these data were not included in the report but are available upon request). Similar but less severe signs were observed at dosages of 560 and 320 mg/kg. Convulsant affects were not observed at these levels. It was also reported that at dose levels of 100 and 180 mg/kg motor coordinating effects were observed in 2 animals and at 180 mg/kg occasional head shaking was observed.

Observations of gross organ pathology were not made at the time of prefusion with the exception of a few animals. However, during necropsy a number of injuries were confirmed, which were regarded as being secondary to the convulsant effects of the compound. The convulsions resulted in the inability of the rabbits to retract their legs from the grid floor while convulsing.

[Body weight changes revealed no significant treatment or sex differences.]

Histological examination of male brain tissues revealed singular and barely perceptible effects at 100, 180 and 320 mg/kg. Examination of peripheral nerve tissue in the same animals revealed easily to readily seen lesions. Brain tissue at 560 and 1000 mg/kg showed no lesions even though peripheral nerves were affected with observations ranging from barely perceptible to extensive involvement. Animals of all dose groups including controls showed lesions. A spinal lesion was observed in one male that received 320 mg/kg. Females showed no lesions in brain tissue or spinal cord for treated groups. Some animals in all dose groups showed lesions of the peripheral nerves.

Weighted scores for sciatic, tibial, and plantar nerves were as follows:

Sciatic, Tibial and Plantar Nerves, Weighted Scores* Incidences and Severity of Nerve Lesions - Rabbits

Dose (mg/kg)	Males	Females
0	0.36	0.08
100	1.12	0.12
180	0.43	0.59
320	0.70	0.07
560	0.33	1.33
1000	0.89	0.40

*$\frac{\text{no. of animals affected x severity}}{\text{no. of animals affected}}$ = score

Examination of the results indicated that in many instances the rabbits which had lesions were also rabbits that were either injured during or following dosing. A correlation between animals showing lesions and those showing trauma appeared to evident.

However, compound related lesions were also evident in treated rabbits. Due to the presence of nerve lesions in control rabbits and possible mechanical injuries throughout the study this compound related effect was not quantified.

Classification: Core-Supplementary