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

009003

CAS. No. 77A

April 3, 1981

OFFICE OF
PESTICIDES AND TOXIC SUBSTANCES

MEMORANDUM

DATE:

SUBJECT: (1) Toxicologic and Carcinogenic Study of SD-43775 by Dietary Administration to Mice for Two Years. (2) A Lifetime Dietary Feeding Study in Rats of SD-43775 at the Singular Dose Level of 1000 ppm.

FROM: Albin B. Kocialski, Ph.D. 3/2/81
Toxicology Branch/HED (TS-769)

TO: F. D. R. Gee, PM #17
Registration Division (TS-769)

THRU: Robert Coberly
Decision Unit Leader
Toxicology Branch/HED (TS-769)

Petitioner: Shell Oil (Chemical) Company
Suite 200
1025 Connecticut Ave., N.W.
Washington, D.C. 20036

Recommendation: It is requested that the Shell Chemical Company respond to the reviewer's questions appearing at the end of each summary and conclusion of the oncogenic studies.

Conclusions: Dietary administration of Pydrin (SD-43775) at 1000 ppm results in the production of spindle cell sarcomas in male rats of the [CRL: COBS CD (SD) BR] strain.

Dietary administration of Pydrin (SD-43775) at 1250 ppm results in no observable oncogenic effects in mice of the B6C3F1 strain.

0001 452

Summary and Conclusion

Mouse Study: Dietary administration of SD 43775 to male and female mice for two years at concentrations of 10, 50, 250 and 1250 ppm caused clearly increased mortality in both sexes only at the highest feeding level, in comparison to untreated controls. Mortality was not dose-related at the lower diet concentrations (the 10 ppm males had nearly the mortality percentage as was found in the highest dose).

Mean body weights were statistically significantly reduced, compared to controls in the 1250 ppm mice (both sexes). While these reductions were fairly consistent throughout the study, body weights of mice in the other groups showed no clear pattern with dose or time, except that the 250 ppm females began to lag and were statistically significantly lower from controls from about the 60th week on to the time of terminal kill.

Tissue masses observed grossly throughout the study were not dose related.

The only clinical chemistry changes observed (serum albumin reduction and SGOT elevation) were in the 1250 ppm group. Hematology findings were not exceptional.

Organ weights and organ-body weight ratios gave no real indications of a dose-relationship.

Dietary administration of Pydrin to male and female mice for two (2) years did not result in statistically significant differences in the number or type of neoplasms, when compared to the incidence of neoplasms in mice fed untreated diet concurrently.

The only non-neoplastic pathology induced by dietary administration of Pydrin was multifocal granulomata. In female mice, this lesion was present in mesenteric lymph nodes, liver, and spleen at dosage levels of 250 and 1250 ppm Pydrin. In male mice; 1250 ppm Pydrin induced multifocal granulomata in mesenteric lymph nodes, other lymph nodes, liver and spleen. Less severe granulomatous lesions were present in mesenteric lymph nodes of male mice administered 50 and 250 ppm Pydrin.

NOEL: Males: 10 - 50 ppm
Female: 50 - 250 ppm

LEL: Males: 50 ppm, multifocal granulomatous lymphadenitis of the mesenteric lymph nodes.

Females: 250 ppm, multifocal granulomatous lymphadenitis (mesenteric lymph node); multifocal granuloma of the liver; small pigmented granulomata in the spleenic white pulp. Statistically lower mean body weights after 60 weeks.

HEL: Males: 1250 ppm, multifocal granulomatous lymphadenitis (mesenteric); multifocal granuloma of the liver; small pigmented granulomata were present in the spleenic white pulp. Increased mortality. Statistically significant reduction in body weight. Increased SGOT. Decreased serum albumin. Increased brain/body weight ratio.

Female: 1250 ppm, multifocal granulomatous lymphadenitis (mesenteric); multifocal granulomata of the liver; small pigmented granulomata were present in the spleenic white pulp. Increased mortality. Statistically significant reduction in body weight. Increased SGOT. Decreased serum albumin. Statistically significant kidney/body weight ratio increase.

Classification: Core-Guidelines

Addendum: Reference: PP 7F2013. Review of Reto Engler dated July 21, 1978.

Pydrin was administered in the diet to d d y strain mice for an 18 month period. Doses administered were 100, 300, 1000 and 3000 ppm.

The results and conclusions of the study using d d y strain mice, which was reviewed by Reto Engler, of the Toxicology Branch are generally parallel to and confirm the results and conclusions reported in the study, using B6C3F1 strain mice, which was reviewed by Albin Kocialski.

The results and conclusions of the two studies, with regard to granulomas, effect levels and oncogenicity were:

1. Oncogenicity is not indicated,

2 granulomatous changes in lymph nodes, spleen and liver were present and related to dose level,

3. the 100 ppm dose level was the lowest effect level (LEL) for effects in d d y strain mice and a No-Observable-Effect-level was not observed,

4 the no-observable-effect-level for B6C3F1 mice was between 10 and 50 ppm.

Reviewer's Questions:

1. Portions of liver and mesenteric lymph nodes from each of five mice per sex from the controls and high dose groups, that were killed at termination were preserved in glutaraldehyde, for special processing, presumably for examination under the electron microscope. The Toxicology Branch would like to know the purpose of the examination the results and the interpretation given to the electron microscopy examination.

2 What statistical method was used to analyze for the statistical significance of tumors?

Conclusion: Two Year Rat Study

It is therefore concluded that:

(1) SD 43775 produces spindle cell sarcomas in male rats as defined by the experimental conditions.

(2) SD 43775 elicits a statistically significant body weight decrease in males and females at the singular dose tested, 1000 ppm.

(3) SD 43775 is the apparent cause of hindlimb weakness in male rats (6/50) which is transient, reversible, and apparently without long-term after effects. The animals showing hindlimb weakness were not those manifesting spindle cell sarcomas.

(4) Interstitial cell tumors are not, in the opinion of this reviewer, compound related.

Classification: Core-Guideline

Synopsis of effects at 1000 ppm - only dose tested.

- Survival rate for males and females was 50-60% at the end of two years.
- Females receiving the test compound began dying 10 weeks earlier than males receiving the test compound.
- Males from week 16 and females from week 44 showed statistically significant and consistent weight loss for the duration of the experiment.
- Daily food intake was comparable for all groups.
- Tissue masses was a generic term encompassing all kinds of masses.
- Reversible hindlimb weakness was evident only in some males, within 12 weeks of compound administration. The effect was compound related.
- Hair loss did not appear to be compound related.
- Hematology for all groups was comparable.
- Urinalysis for all groups was comparable.
- Clinical chemistry - values for SGOT glucose, Na and K differed from controls but no definitive conclusions were made as to their significance.

- o General protein electrophoresis indicated a raised gamma globulin value for females, but the relationship to the compound is uncertain.
- o Organ weights were comparable for all groups.
- o Spindle cell sarcomas were statistically significant in males and compound related.
- o Interstitial cell tumors were evident in males but not compound related.

Reviewer's Questions:

1. Please provide the Agency with a rational for the selection of the particular strain of rat [CRL: COBS CD (SD) Br] utilized in this study.
2. Please provide the Agency with a rationale for the selection of the dose level used in this study.
3. The protocol states that the animals were observed daily for mortality and/or a moribund condition during the first year of the study and once every four weeks the animals were examined clinically. Please provide any other data, previously considered not pertinent (if such circumstances existed), regarding the onset, description, severity and duration of the animal sign "hindlimb weakness", in the animals observed in this study. This Toxicology Branch reviewer finds it quite surprising and remiss on the part of the sponsor and contractor that this effect was not given the due attention it deserved.

OPP:HED:TOX: A.KOCIALSKI:sb 2/27/81 CM 2 Rm. 820 X77395 #7R

Test: Lifetime Feeding Study in Rats

EPA Acc. No. 241208

Test Initiated: December 30, 1976

Test Terminated: January 2 and 5, 1979

Report Submitted to Sponsor: October 1979

Report Lab. No. LBI Project No. 20733-01

Facility: Litton Bionetics Inc.
5516 Nicholson Lane
Kensington, Maryland 20795

Submitted by: Elliot B. Gordon, Ph.D.
Robert P. Belilles, Ph.D.
Robert J. Weir, Ph.D.

Chief Pathologist: William C. Hall, D.V.M., Ph.D.
Richard H. Cardy, D.V.M.

Title: Lifetime Feeding Study in Rats.
SD-43775 Technical.

Purpose:

The purpose of this study was to further evaluate and characterize the chronic toxicity of SD-43775 at a previously untested high dose level. This study is to complement an earlier two year rat chronic toxicity study [LBI Project No. 20541; Toxicology Branch Reference Caswell #77A; PP 7F2013; Acc. No. 097075-097082; see Review by Dr. Larry Anderson July 17, 1978 LBI Proj. No. 2541 (Sic ?)]

Materials and Methods:

Twenty-one-day old outbred albino rats [CRL: COBS CD (SD) Br; Charles River Breeding Laboratories, Wilmington, Massachusettes] were acclimated for nine days, prior to dosing, to the laboratory conditions in the Falls Church, Virginia facility of Litton Bionetics. The animal rooms were within the clean-dirty barrier system. The annual room temperature ranged between 71 and 77° F, and a 12 hour light-dark cycle was maintained. There were no other compounds on test in the rooms used for this study, other than a separate and concurrent study (LBI Project No 20733-02) in SD-43775. The test diet was prepared by dissolving 10 grams of 98% pure technical SD-43775 in acetone to make a 25 ml. solution and then blending this solution with standard Purina Laboratory Chow meal (5001) for each 10 kg of feed prepared. [Initially from December 30, 1976 to January 26, 1977 the solvent in the diet preparation was hexane. The change reflected the concern of possible peripheral nerve effects due to use of hexane.] The mix was achieved by first blending the solvent/test material solution with a mortar and pestle into a few hundred grams of the basal diet and then adding this premix to a Patterson-Kelly twin shell blender equiped with an intensifier bar, which contained approximately one-half the final volume of feed. After blending for 15 minutes, the remaining half of the feed was added and the mixture was blended again for another 15-20 minutes. This procedure resulted in a blend of 1000 ppm.

The control diet was prepared by mixing 10-15 ml of solvent into the chow meal for each kilogram of diet.

The control and experimental diets were analyzed five times between November 10, 1977 and October 25, 1979, for the presence and concentration of SD-43775. All the analytical results were within 10% of the nominal concentration.

The standard Purina Chow meal (5001) diet was also periodically analyzed for the presence of cholrinated pesticides, aflatoxins, metals and antibiotics.

The heat treated, hardwood chip bedding (AB-SORB-DRI) was alos periodtcally analyzed for polychlorinated biphenyls and pentachlorophenol.

Tap water, prior to acidification, was sampled on August 15. 1978 for conformance with EPA's interim drinking water standards. The purpose of acidifiying the tap water is to keep the level of Psuedomonas spp (bacterial organisms commonly found in tap water) low.

The results of the analysis for bedding, feed and water are appended to this review

Twenty-one day old outbred albino rats [CRL: COBS CD (SD) BR; Charles River Breeding Laboratories] were divided into two groups of 50 animals per sex per group. The experimental group received 1000 ppm of SD 43775 in the diet whereas the controls were treated similarly but had the test chemical excluded from their feed. The animals were initially housed for a one month period three per cage in suspended polycarbonate cages with AB-SORB-DRI bedding and later two per cage. The relative position of the cages on the rack remained constant until week forty-one (41) of the study. After week 41 the rows of cages were moved approximately monthly in a regular sequence (i e. top row to the bottom, second row from the top to the top row, etc.). All animals received bottled acidified (ph 2.5) tap water and their respective diets ad libitum. The animals were observed daily for mortality or a moribund condition for the first year and a half of the study and thereafter twice daily. Body weight for each individual animal was taken once every four weeks. Food intake data were collected once every four weeks on twenty percent of the animals (first five cages of each group) Once every four weeks the animals were examined clinically and palpated for masses.

Animals dying intercurrently (found dead) or judged to be moribund and killed were necropsied Surviving animals were killed at termination of the study (January 2, 1979 through January 5, 1979) One day prior to necropsy, the animals were fasted and urine was collected overnight with the use of stainless steel metabolism cages. The parameters evaluated were:

Color
Appearance
Specific gravity
pH
Protein (presence of albumin)
Glucose
Ketones
Bilirubin
Occult blood
Microscopic examination of sediment

After urine samples were collected, blood was collected by use of the orbital sinus bleeding technique for the following clinical chemistry (serum) determinations:

Bilirubin
Urea Nitrogen
Albumin
Chloride
Cholesterol
Creatine phosphokinase
Creatinine
Serum glutamic-oxaloacetic transaminase
Alkaline phosphatase
Phosphorus
Total protein
Globulin
Albumin/globulin ratio
Potassium
Sodium
Uric acid
Calcium
Glucose

After sufficient blood was obtained for the clinical chemistry analyses, the animals were administered uthol (pentobarbital, sodium, 5 g/cc). As soon as each rat lost consciousness, the animal was opened and sufficient blood was collected from the aorta for the following hematologic tests:

Red blood cell count
White blood cell count
Differential leukocyte count
Hematocrit
Platelet count (adequacy)
Sedimentation rate
Prothrombin time
Hemoglobin
Clotting time

Following blood withdrawal for the hematology tests, the animals were necropsied by pathology prosectors under the supervision of William Hall, V.M.D., Veterinary Pathologist. During the necropsy, organ weights were taken on the following organs:

Brain
Heart
Liver
Testes
Spleen
Adrenal glands
Kidneys

Suitable samples of the following organ/tissues from the animals killed terminally, as well as those dying intercurrently, were preserved in 10% neutral formalin, and were then trimmed, processed and microslides prepared at 4-6 microns. Tissue sections were primarily stained with hematoxylin and eosin. However, selected sections of sciatic nerve were stained with luxol fast blue for myelin and sections of subcutaneous spindle cell sarcomas were stained to demonstrate collagen, reticulum, and mucin. The following tissues were examined by William Hall, D.V.M.:

Brain	Small intestine (three sections)
Pituitary gland	Large intestine
Eyes, if abnormal	Adrenal gland
Salivary gland, submaxillary	Kidneys
Thyroid gland	Lymph nodes (mesenteric)
Parathyroid gland (when present in plane of section)	Bladder
	Prostate gland
Larynx	Testes/ovaries
Trachea	Uterus
Esophagus	Fallopian tubes
Thymus	Skin (back)
Mammary gland	Skeletal muscle (thigh)
Heart	Bone with marrow (femur)
Lungs	Spinal cord
Liver	Peripheral nerve (sciatic)
Spleen	Pancreas
Stomach	Nasal cavity

The statistics for the various parameters measured, as appropriate, were compared using the Dunnett's t-test or Chi-square test. Values differing from the control values were considered significant when $p < 0.05$.

Results

Cumulative Mortality and Early Female Deaths: The percent cumulative mortalities for controls of both sexes as well as treated males were similar. Treated females showed a slightly higher cumulative mortality, than either males or controls. However, females were not statistically different from control females and generally appeared to be within the range of cumulative mortality for all groups. The treated females did however die somewhat earlier than the other groups, but the rate of death appeared to be similar for all groups. The total number of deaths at termination of the study is shown below by category of death.

Dose Level (ppm)	Sex	Found Dead	Moribund Killed	Subtotal Found dead/ Moribund	Terminal Kill	Total
Control	Male	13	10	23	27	50
Control	Female	3	18	21	29	50
1000	Male	8	13	21	30	51*
1000	Female	7	17	24	25	49*

* One animal mis-sexed.

An inspection of the graphs representing percent cumulative mortality with respect to time for both males and females indicates the initial period for the uniform rate of death was as noted below:

Beginning Period for Uniform Death Rate

Dose	Male	Female
0	64 weeks	86 weeks
1000 ppm	80 weeks	70 weeks

It can be seen that females receiving compound began dying approximately 16 weeks earlier than their own controls and 10 weeks earlier than males receiving test compound. Males receiving 1000 ppm began dying later than their controls only because their own controls started dying relatively early. However, had male controls lived longer (as one would normally expect) or as long as female controls, then the males on test compound would have started dying approximately the same time as their own controls

Body weight: Males showed a consistent decrease in body weight which was statistically significant from week 16 thru 104. Males therefore sustained a 64 week time period of lowered body weight before they began dying with any regularity (week 80 when animals began dying with regularity minus week 16, time period of statistically significant weight decrease). Females showed a consistent decrease in body weight which was statistically significant from week 44 thru to week 104. Females therefore sustained a 26 week period of decreased body weight before they started dying with any regularity (i.e. 70-44 weeks = 26 weeks).

Females, therefore, began losing weight later than males but began dying off earlier, and males began losing weight sooner than females but began dying later than females.

It would therefore appear that males although affected earlier were better able to tolerate (thru various possible appropriate mechanisms) the chemical, whereas females while able to tolerate the chemical in the early stages of its administration succumed earlier once the tolerance mechamism was overwhelmed.

However, to reiterate, both sexes began dying off at a uniform rate once the mechanism(s) for handling the xenobiotic were dissipated.

Daily Food Intake: The daily food intake between test and control animals was comparable with no significant differences noted for males during the duration of the experiment. There was also no statistically significant difference between females and their own respective controls. Therefore, the weight lost by the test animals can not be attributed to decrease feed consumption.

Tissue Massses: Tissue masses were described as small, medium and large. Papillomas were also included in the category "tissue masses" but were described separately. However, the total number of masses reported included all palpable masses and were not necessarily tumors. Included in the category of masses were such biological manifestations as active mammary tissue, scar tissue formed by cutaneous lesions, masses due to salivary gland infection, and local infections.

The number of male and female animals receiving test compound, and showing at least one mass, was not significantly different from their own controls. The total number of masses (including multiple masses per animal) between males on test and control males were comparable.

The total number of masses including multiple masses per animal between females on test and control females indicated that control females had 1.5 times more masses than the females receiving the test compound.

Therefore, an arguement can be made that the early deaths in the females, which was noted earlier in this review, were in part caused by compound administration and not solely to the presence of the size, number and type of masses. The size, number and type of masses in females did not appear to be treatment related in females (or males).

Hindlimb Weakness: Six male rats receiving compound showed hindlimb weakness within eight weeks of the initiation of the experiment. Hindlimb weakness was not seen in females. This weakness was characterized by an abnormal gait. The rear legs would appear to falter, and the rat would compensate for this weakness by using its front legs to pull itself along the floor of the cage. It was judged by the authors of the report that this condition was related to administration of the test material.

The male animals coded 7185, 7186, 7197, 7198 manifested hindlimb weakness during the third week of observation. Hindlimb weakness was not observed at week four or during any later period for these four animals. The effect can be considered transient and reversible. Two of these animals were sacrificed at the terminal kill and two were found dead at 105 weeks.

Animals numbered 7194 and 7203 manifested hindlimb weakness at the fourth week of observation. Both animals survived the experiment to the terminal kill. Animal 7194 showed no signs of hindlimb weakness before or after week four, however, the animal numbered 7203 showed evidence of hindlimb weakness at week eight, but not at the next observation period which was week 12. The onset and duration of signs were difficult to estimate due to the time intervals of the observation periods.

The examination of other parameters including histopathology for each of the above animals gave no readily acceptable cause for the hindlimb weakness nor any readily available hypothesis for the observed effect. Additionally, hair loss for each of the above animals did not appear to correlate with hindlimb weakness.

Hair Loss: Male and female animals both control and test animals (all dose levels) were compared for hair loss by this reviewer. The time periods arbitrarily chosen by this reviewer were 26, 50 and 98 weeks.

Male control rats showed no hair loss at the arbitrary selection period of 26 weeks. Only one male control rat showed some hair loss at 50 weeks (animal #7121) behind the right ear. Only one animal (#7128) showed some slight hair loss behind the left ear. All these animals survived to the period of the terminal kill.

Seven males on test at 26 weeks showed some hair loss. Five of the animals survived the duration of the experiment. At 50 weeks eight animals showed some hair loss and seven of these survived the experimental duration. Five animals showed some hair loss at 98 weeks and all five were sacrificed at the time of terminal kill.

Three of five animals showing hair loss at 98 weeks were also the same three animals that showed hair loss at 26 and 50 weeks. Animals numbered 7214, 7226 and 7229 generally showed a relatively stable alopecia at the three time periods. Some of the animals in this arbitrary sample that showed hair loss at either 26 or 50 weeks did not show hair loss at succeeding time periods. Furthermore, hair loss was almost always confined to the upper part of the body of these animals.

It is also noted that of all the test animals recorded as showing hair loss in this arbitrary sample, only three died inter-currently.

It is the opinion of this Toxicology Branch reviewer that for males fed 1000 ppm of test compound, for this arbitrary selection of time periods it appears that for this sample:

1. Hair loss is not correlated with death of the animal.

2. Hair loss is apparently not time dependent in that the number of animals showing alopecia does not increase with time, and that the severity of the alopecia does not appear to increase with time.

3. Hair loss in some instances was reversible.

Control females and female rats receiving the test compound, when examined for weeks 26, 50 and 98, generally showed quantitative and qualitatively similar responses. The conclusions arrived at for males are therefore also applicable here for females. In those instances of recorded generalized hair loss (98 weeks), the hair loss was usually accompanied by some sort of large tissue mass.

Generally speaking for both male and female test animals as most of the alopecia was confined to the upper body, one might speculate that at least in some cases, hair loss may be due to animals fighting, or some other non-compound related cause. Age as related to alopecia should also not be ruled out in some cases.

Hematology: Males. Reported values for males receiving the test compound were comparable to controls. Females showed comparable values between those receiving the test compound and those not receiving the test compound for all categories of measurement except two; hemoglobin and packed cell volume were significantly lower than control values. It is however difficult to accept these results at face value for the following reasons - packed cell volume is essentially a function of the formed elements of the blood. The results of the measurements of individual formed elements showed comparable values between test and control animals. Therefore, as the individual formed elements of the blood are comparable to controls, packed cell volume should also be comparable between animals receiving test compound and their own controls. The lower hemoglobin value in test animals is not affirmed by the ancillary data within the report. The test animals were not reported as suffering

from dyspnea. Pathology and organ weight data did not indicate cardiac compensation as manifested by an enlarged heart. The red blood cell count was normal as were bilirubin levels. The histopathological examination of bone with marrow was also reported as normal. Additionally, review of the data does appear to support a conclusion for hemodilution.

Therefore, based upon the report it would appear that the lowered values for packed cell volume and hemoglobin might be attributed to some other cause rather than to the administration of the test compound.

Clinical Chemistry: Serum-glutamate-oxalate - transaminase levels, in females were statistically significantly decreased when compared to control values. However, as one would normally expect an increase in these levels as a manifestation of a toxic effect the reported values are therefore not considered by this reviewer as being biologically meaningful, and most likely are not a result of compound administration.

The significantly increased glucose levels in male rats may or may not be compound related. Sodium levels were statistically significantly lower in females, whereas, potasium was statistically significantly elevated in males. The results for both these alkali metals may be artifact (chloride values were comparable for test and controls) or related to the compound's effect upon the kidney. However, other possibilities may exist for glucose, sodium and potassium when considered together, such as adrenal and/or pitiutary gland involvement.

Urinalysis: Inspection of each of the parameters measured did not reveal a compound-related effect when the test groups were compared to their respective controls.

Protein Electrophoresis: Protein electrophoresis was conducted on serum protein and numerical values were determined for total protein, albumin, alpha-1-globulin, alpha-2-globulin, beta globulin, gamma globulin and the albumin-globulin ratio. Male rats showed no significant differences between treated and control animals for the values recorded. Female, showed no significant differences between values from treated and controls with the exception of a statistically significant increase in gamma globulin values for treated groups (8.69% for controls vs. 12.24 for treated). However, there does not appear to be a readily available explanation for these results from the data available. Parenthetically, however, viral or bacterial invasion should not be ruled out, as a possible explanation, nor the compound itself which may act as an antigen, and therefore stimulate the antibodies. Experimental error should also not be ruled out.

Organ Weight: The actual (absolute) organ weights of the treated animals were similar to controls. The organ weight - body weight percentages (i.e. ratio) however were significantly higher in males for the brain, liver, heart, spleen and testes and in females for the brain, kidneys, liver, spleen and heart. This increase was due to the lowered body weight of treated animals when compared to controls. The increased organ weight - body weight percentages were therefore judged to be related to treatment with the test material, but of no toxicologic significance.

Pathology: The final pathology report made mention of the fact that interstitial cell testicular tumors were found in 5 of 50 male rats receiving the test compound but only 1 of 50 control animals manifested this tumor. The author of the report further stated that although the incidence of this lesion was low in the control group of this study, interstitial cell tumors were found in 5 of 80 Sprague-Dawley rats used as controls of a similar age and reared at Litton Bionetics, from a separate and previously conducted study (see summary pages of final pathology report pp. 00280-00284; Accession No. 241208). It was then concluded that the lesions were not compound related.

This Toxicology Branch reviewer does not agree with the rational for the conclusion presented by the author for these two reasons: (1) it should be noted that even though the incidence in these connective tissue type tumors are numerically low, no evidence was presented as to their statistical significance or non-significance. The level of significance or non-significance should have been established and the statistical method referenced, and (2) if a matching control group is available historical controls can not be used. The rationale presented by the sponsor would obviate the need for concurrent controls and eventually lead to a host of problems of interpretation of data submitted in future studies.

This Toxicology Branch reviewer does however agree with the conclusion of the author in that the interstitial cell tumors are probably not compound related. It is generally known that interstitial cell tumors are common in aging rats. One aging rat of the control group was found to have an interstitial tumor, at the time of the terminal kill. The terminal kill occurred at about 24 months after the initial compound administration and coincided with the ending of the normal life-span of the rat. Five rats of the treated group were found to have interstitial cell tumors. Three of the rats had interstitial cell tumors as diagnosed at the time of terminal kill. Two of the five rats which showed interstitial cell tumors died intercurrently. However, these two animals, 7199 and 7213,

died on November 21 and August 11 of 1978. The terminal kill took place between January 2 and January 5, 1979. Therefore these two rats were by ordinary standards old rats. Interstitial cell tumors were not found in other males dying intercurrently. Therefore, interstitial cell tumors do not appear to be compound related based upon what is generally known and the results of this experiment. Additionally, Bert Litt, the Toxicology Branch statistician provided a statistical analysis of the one tumor found in the controls versus the five tumors found in the treated group, using the Fisher's Exact Test. Mr. Litt's conclusion was that the incidence in the treated group was borderline as to its statistical significance, with a "p" value equal to or less than 0.0913.

Therefore it is the belief of this Toxicology Branch reviewer that the interstitial tumors in male rats are probably not compound related because the tumors are (1) not uncommon in aging male rats and (2) the statistical significance is borderline as judged by Mr. Litt.

The author of the report also concluded that the spindle cell sarcomas that were observed in the subcutis and dermis of 5 out of 51 treated males (9.8%) but not in the concurrent (matched) controls (zero out of 50) only suggested compound relatedness.

Animals Showing Spinale Cell Sarcomas

Terminal Kill	Animal No.	Time
January 5, 1979	7224	24 months
January 5, 1979	7230	24 months
Moribund Kill	**Animal No.**	**Time**
October 18, 1978	7188	22 months
August 11, 1978	7213	20 months
Natural Death	**Animal No.**	**Time**
November 23, 1977	7227	11 months

The author stated that the historical data from the Archies at Litton Bionetics and information from the literature indicated that the spindle cell sarcomas were observed more frequently in control male rats of Other reported studies in animals of this strain and age than were observed in the controls of this reported study. In those other studies (refer to pp. 00282 of Accession No. 241208) the incidence of such lesions ranged from zero to 6%, thus indicating a possibility of the chance occurrence of spindle cell sarcomas in test animals of this study. The author of the pathology report concluded that the results of this single dose study were equivocal but not clearly negative.

This Toxicology Branch reviewer disagrees with the conclusion in this section of the report for the following three reasons: (1) historical controls can not be used if matched concurrent controls are available (please refer to this reviewer's opinion on the use of historical controls which appears earlier in this discussion), (2) it is noted that even though the incidence of these connective tissue type tumors was five no evidence was presented as to their statistical significance, and (3) a statistical analysis using the Fisher's Exact Method was conducted by Mr. Bert Litt, the Toxicology Branch Statistician, using data as reported for spindle cell sarcomas. The Toxicology Branch statistician, Mr. Bert Litt concluded that the tumor incidence was significant at a "p" value equal to or less than 0.027 (see attached).

It is therefore concluded by this reviewer that the test chemical SD43775 and/or one or more of its metabolites is the apparent causative agent of spindle cell sarcomas under the conditions of the experiment and available evidence but is apparently not related to the incidence of interstitial cell tumors which are probably related to the natural aging of the animals.

Sparately the pathology report also stated that the neoplasms of the anterior pituitary gland were the most frequently occurring tumor of the study. Anterior pituitary tumors were observed in 59% of the male controls and in 59% of males receiving the test compound. Anterior pituitary tumors were also found in 67% of the control females and 67% of the females receiving the test compound. These percentage values are correct as reported. This reviewer conducted an individual count of all animals and noted which individual animals manifested anterior pituitary gland tumors. The results of this survey are noted below:

Anterior Pituitary Tumors

Males:	Control	Test
	28/48 = 58.3%	30/51 = 58.8%
Females:	Control	Test
	33/49 = 67.3%	33/48 = 68.7%

The numerator indicates the number of animals identified by the pathologist as having anterior pituitary tumors.

The denominator indicates the total number of animals (i.e. tissue) examined.

It is also interesting to note here that of the five male rats which manifested interstitial cell tumors, only two of these animals (#7199 and 7219) had anterior pituitary tumors. The three remaining animals (#7214, 7213 and 7214) which manifested interstitial cell tumors did not show any apparent evidence for a pituitary tumor even though interstitial cell tumors were diagnosable.

Conclusion:

It is therefore concluded that:

(1) SD 43775 produces spindle cell sarcomas in male rats as defined by the experimental conditions.

(2) SD 43775 elicits a statistically significant body weight decrease in males and females at the singular dose tested, 1000 ppm.

(3) SD 43775 is the apparent cause of hindlimb weakness in male rats (6/50) which is transient, reversible, and apparently without long-term aftrer effects.

(4) Interstitial cell tumors are not, in the opinion of this reviewer, compound related.

Classification: Core-Guideline

OPP:HED:TOX: A.KOCIALSKI:sb 12/17/80 X77395 TS-769 Rm. 820 CM 2 #8

Synopsis of effects at 1000 ppm - only dose tested.

- Survival rate for males and females was 50-60% at the end of two years.
- Females receiving the test compound began dying 10 weeks earlier than males receiving the test compound.
- Males from week 16 and females from week 44 showed statistically significant and consistent weight loss for the duration of the experiment.
- Daily food intake was comparable for all groups.
- Tissue masses was a generic term encompassing all kinds of masses.
- Reversible hindlimb weakness was evident only in some males, within 12 weeks of compound administration. The effect was compound related.
- Hair loss did not appear to be compound related.
- Hematology for all groups was comparable.
- Urinalysis for all groups was comparable.
- Clinical chemistry - values for SGOT glucose, Na and K differed from controls but no definitive conclusions were made as to their significance.
- General protein electrophoresis indicated a raised gamma globulin value for females, but the relationship to the compound is uncertain.
- Organ weights were comparable for all groups.
- Spindle cell sarcomas were statistically significant in males and compound related.
- Interstitial cell tumors were evident in males but not compound related.

Questions:

1. Please provide the Agency with a rational for the selection of the particular strain of rat [CRL: COBS CD (SD) Br] utilized in this study.

2. Please provide the Agency with a rationale for the selection of the dose level used in this study.

3. The protocol states that the animals were observed daily for mortality and/or a moribund condition during the first year of the study and once every four weeks the animals were examined clinically. Please provide any other data, previously considered not pertinent (if such circumstances existed), regarding the onset, description, severity and duration of the animal sign "hindlimb weakness", in the animals observed in this study. This Toxicology Branch reviewer finds it quite surprising and remiss on the part of the sponsor and contractor that this effect was not given the due attention it deserved.

Pages 22-23 - * Claimed confidential by submitter*

Fisher's Exact Test 13-

To Albin Kocialski

Re Pydrin (SD 43775); Fenvalerate Sumicidin (S-5602, #24377)

	100 ppm Treated	0 Controls	All
♂ Spindle Cell Sarcomas	5	0	5
♂ Free of "	46	50	96
# Examined	51	50	101

Exact Test

$\frac{5!\,50!\,51!\,96!}{5!\,46!\,50!\,101!} = .026566$

The probability of 5 sarcomas or fewer in 51 Treated animals compared with zero of 50 controls .026566 as the result of chance alone, i.e. P = .027

	100 ppm	0	
Interstitial Cell Tumors ♂	5	1	6
♂ Free of alone	45	49	94
# Examined	50	50	100

$\frac{6!\,50!\,50!\,94!}{5!\,45!\,44!\,100!} + \frac{6!\,50!\,50!\,94!}{6!\,44!\,50!\,100!} =$

$.03887 + .00248266 = .09133$

The probability of observing 5 cases of Interstitial cell Tumor cells in 50 rats treated compared with 1 of 50 controls is .0913 as the result of chance alone, i.e. P ≤ .0913

Thus this is a statistically significant finding (P = .026) of more spindle cell sarcomas in male rats than expected and borderline finding of increased frequency of interstitial cell tumors (P = .0193) in male rats.

[signature] 11/3/80

BEST AVAILABLE COPY

Addendum:

This Toxicology Branch reviewer includes the following comments with regard to the author's use of historical data. It is included as an aside from the official review but not necessarily excluded from the review itself.

The author uses the phrase.. "Sprague-Dawley rats of similar origin".... This statement is to some extent true in that all Sprague-Dawley rats had a common origin. However, there are probably several breeders (suppliers) of Sprague-Dawley rats, each with its own currently evolved line of genotype. Although all the lines may be relatively similar, genetic variation exists between the lines thereby dictating a possible range of responses between genetic lines (pharmacogenetics). The direct comparison of responses between different genetic lines of the same strain separate by time, space and experimental protocol would therefore not appear to be appropriate. The author's statement can therefore be considered vague and some what misleading. The impression given is that similar lines are identical (i.e. no genetic difference between lines) and therefore one can expect an identical response between genetically evolved lines. This is not necessarily true.

The author also refers to several previous studies conducted at Litton Bionetics, the National Cancer Institute and the open publised literature for support of his position that the spindle cell sarcomas are not uncommon in this strain of rat and, that the incidence in the test sample is comparable to those of previous historical controls. The author stated that the incidence of spindle cell sarcomas ranged between zero and six percent. If one lists the frequency (f) of spindle cell sarcomas for the five referenced studies conducted at Litton Bionetics, Inc. (LBI) one obtains the following:

Reference		"f"	Percent
LBI	Project No. 20541	2/102	2%
LBI	Project No. 20823	2/50	4%
LBI	Project No. 20876	0/50	0%
LBI	Project No. 20584	0/64	0%
LBI	Project No. 1400	0/83	0%

The range of responses for spindle cell sarcomas is from 0 to 4% for these individual studies. This is one way of examining the data. However, noting what has previously been stated in the earlier paragraphs, it would appear that a better representation of the population incidence for spindle cell sarcomas could be obtained by summing up the individual incidences and the population size for each experiment. If one looks at the data in this manner then the frequency of response would be 4/349 which is equal to about 1.14%.

If one totals up the incidences of spindle cell sarcomas for the four literature references one obtains the following:

Reference	"f"	Percent
MacKenzie and Garner, 1973	4/535	0.74
Thompson et al , 1969	1/16	6.20
N. C. I. Bioassay	1/215	0.50
N. C. I. Bioassay	1/25	4.00

If one adds up the total incidences for the four references the incidence becomes 7/791 equivalent to 1.0%.

A total of all the incidences for all the historical referenced data (present study excluded) reveals a ratio of 11/1140 for a percentage incidence of 1.0%.

It would therefore appear that as the population sample gets larger the natural incidence becomes smaller (i.e. it tends towards a 1.0% incidence or less) and therefore the test group (i.e. males receiving SD 43775 at 1000 ppm in this reported experiment) having an incidence of 5/51 equivalent to 9.8%, compared to zero incidence for concurrent controls appears significanly increased from the aggragate population as a whole. The caveat however is that we are probably comparing different genetic lines of the same strain and therefore some room for error may exist, but still in all, by adding up all the individual population groups the differences between the various groups may well have been greatly diminished and in turn would support the Toxicology Branch position that the incidence of 5/51 spindle cell sarcomas, 9.8% of the sample size, is both biologically and statistically meaningful.

If we are not comparing different lines of the same strain, but the same genetic lines of the same strain (i.e. animals not separated by time, space, and experimental protocol), then the data proposed by the author of the pathology report would also appear to support the position of this Toxicology Branch reviewer.

It is also known that the results of acute studies (Weil and Scala - reference not known) between laboratories for the same chemical compound vary greatly. If one extends the concept of the variability of results between laboratories for acute studies to chronic studies, then the number and magnitude of the variables and the variable animal responses can easily be evisioned.

Title: Toxicologic and Carcinogenic Study of SD-43775 by Dietary Administration to Mice for Two Years.

EPA Accession: #241206-241207

Test Initiated: February 17, 1977

Test Terminated: February 1979

Report Lab No.: L.B.I. Project No. 20738

Facility: Litton Bionetics, Inc.
5516 Nicholson Lane
Kensigton. Maryland 20795

Submitted by: Carter D. Johnston, Ph.D
Robert P Belliles, Ph.D
Robert J. Weir, Ph.D.

Pathology report submitted separately from Litton's report but jointly by Westhollow Research Center, Shell Development Company, Houston Texas; C. B McCullough, D.V.M., Ph.D. -- examined only tissues from male mice, and Shell Toxicology Laboratory (Tunstall), Shell Research Ltd , Settingbourne, Kent, England. J.B.M. Gellatly. B Sc., M.R C.V.S. -- examined only tissue from female mice

Subject: Two Year Mouse Oncogenicity Study Using B6C3F1 Mice. Oral Feeding.

*Materials and Methods

Male and female B6C3F1 mice (Fredrick Cancer Research Center, Fredrick, Maryland) five to seven weeks of age were divided into six groups of 50 males and 50 females per dose group. The groups were formed on a body weight basis. All animals were individually identified by toe clipping. Groups one and two served as two separate and independent vehicle control groups. Groups three, four, five and six received respectively 10, 50, 250, and 1250 ppm of Pydrin (SD 43775) in their feed.

Mice were housed in plastic cages on a hardwood chip bedding (AB-SORB-ORI) five per cage per sex. Food (Purina Laboratory Chow, meal) and acidified tap water (ph 2.5) were available ad libitum. Note here that acidification of water aids in the control of Psyedomonas micro-organisms normally found in tap water.

Inspection for mortality was made twice daily, five days per week and once daily on weekends. Gross signs of toxicity (if any) were noted daily and a careful physical examination of each mouse was made weekly and included palpation for tissue masses. Animals in poor health were transferred to an individual cage for observation.

Individual body weights were recorded initially and then monthly. Food consumption was estimated on a monthly basis. Additionally five (5) male control mice were bled from the orbital sinus at week 26 and the samples submitted for serological testing for hepatitis. (The results were negative.)

Hematology and clinical chemistry tests were conducted at termination on 6 control mice per sex per group and a minimum of 12 mice per sex per treated group. The hematological parameters evaluated were, hematocrit, erythrocyte count, total and differential leucocyte counts and hemoglobin. The clinical chemistry parameters evaluated were, BUN, glucose, albumin, SAP, and SGOT.

The following organs were weighed from the same mice from which the blood samples were drawn for hematology and clinical chemistry; they were brain, heart, liver, kidney and adrenal glands.

A gross necropsy was performed on each animal surviving at termination. Mice that died on study or were killed in a moribund condition were also necropsied. When tissue conditions permitted, the following organs (or portions thereof) were preserved in 10% buffered formalin to be processed for histopathologic examinations--brain, pituitary, salivary gland, thyroid gland, larynx, trachea, esophagus, thymus, mammary glands, large

* 98% Technical

intestines, adrenal glands, kidneys, lymph nodes (mesenteric, dermal, mandibular) urinary bladder, prostate gland, testes, ovaries, uterus, heart, lung, liver, spleen, pancreas, stomach, small intestines, fallopian tubes, parathyroids, epididymides, skin (dorsal) skeletal muscle, bone with marrow (femur), nasal turbinates, spinal cord (thoracic) and peripheral nerve.

Separately and in addition to the above, portions of liver and lymph node from each of five mice per sex from the controls and high-dose groups that were killed at termination were preserved in glutaraldehyde for special processing (presumably for electron microscopy examination).

The slides prepared at Litton Bionetics were then delivered to representatives of Shell Chemical Company for examination and interpretation. All tissues from male mice were examined by C. B. McCullough of the Shell Company, Houston, Texas. All tissues from female mice were examined by J. B. M. Gellatly at the Shell Toxicology Laboratory (Tunstall), Kent, England. The pathology report was then compiled jointly by the responsible pathologists.

Statistics employed were the Chi square ($p < 0.05$ as the level of significance) in the analysis of mortality data. The Dunnett's t-test was used in the analysis of effects in treated groups against pooled control values, for at least chronic but apparently not tumorigenic effects.

Diet - Preparation and Analysis

Diets were prepared by adding appropriate volumes of an acetone solution of SD 43775 (40% w/v) to 10 kg (initially) or 20 kg of basal diet and mixing for 30 minutes in a twin-shell blender. Control diet was mixed with the same volume of acetone as that used in preparation of the highest dose level. Diet mixes were prepared at 1-4 week intervals during the first two months of the study and bi-weekly thereafter. Dosing started February 17, 1977 and continued for 104-105 weeks. Samples of each diet concentration mix, including control, were analyzed periodically beginning in November, 1977 and then at 3-5 month intervals. Analysis of the dietary apparently ended during the month of December 1978.

Analysis - Food, Water, Bedding

The standard Purina Chow meal (5001) diet was periodically analyzed for the presence of certain chlorinated pesticides, metals, antibiotics and aflotoxin.

The heat-treated, hardwood chip bedding was periodically analyzed for polychlorinated biphenyls and pentachlorophenol.

Tap water (prior to acidification) was analyzed for conformance with the Environmental Protection Agency's interim drinking water standards.

Results - Analytical

Diet Concentration - Results indicated that the diet concentrations of SD 43775 (Pydrin), with one exception, were between 92 and 102 percent of the nominal concentrations. The one sample exception was at a dose level of 250 ppm of Pydrin in the diet mix which analyzed as five parts per million. The value of five parts per million was subsequently confirmed with a repeat analysis of the same sample. This error in the opinion of this reviewer was short lived (probably only one mixing which provided feed for a two week period - note here that diet was prepared once every two weeks according to the protocol) and would not be expected to affect the integrity of the study. This can be said with some degree of confidence based upon acceptance of the following rationale, which uses female body weight data as the crux of the arguement.

A dietary sample of test feed at the 250 ppm level was taken in July 1978 for an analytical determination and was found lacking by 245 ppm. The last previous analytical determination was made with a sample taken in April of 1978. The time difference between samplings was approximately three months (12 weeks) and constituted the maximum possible time the error could have continued. April represented 56 weeks and July represented 68 weeks into the chronic feeding study.

Body weights of the male mice were comparable to controls for the entire experiment. The body weights of female mice, however, showed a continuous and statistically significant decrease between weeks 60 and 92. A significant weight decrease was also noted at week 52 but not week 56 of the study. It is reiterated here that one preparation of the dietary provided enough feed for two weeks. The fact that a statistically significant decrease in the body weight of females began at week 60 and was maintained thru week 92 of the experiment would seem to eliminate the weeks 60 thru 68 (eight weeks) as a period of error in the preparation of the diet. This would leave only the time period between week 56 and 60 (four weeks) unaccounted for dietary error. This would therefore leave only a minimum of two or a maximum of four weeks open to question as diet was prepared every two weeks.

It can therefore be reasonably concluded that the error in dietary preparation was short lived and did not effect the basic intergrity of the study.

Food, water, bedding - analysis - It was reported in a summary paragraph that review of 32 feed analysis and 29 bedding analysis conducted on material received from March 1977 thru December 1978 indicated relatively uniform background levels of the contaminants selected for analysis. Generally, the bedding was analyzed only for pentachlorophenol and polychlorinated bi-phenyls. The levels found were reported as not exceeding those listed in attachment No. 1.

The results of the drinking water analysis were judged to be satisfactory. These results are reflected in attachment No. 2.

Results

(Chronic Study in Mice)

Survival and Dose Response Mortality: Six hundred mice began the study. Two were accidentally killed and 12 escaped. One hundred-eighty-seven mice died and 23 were killed in a moribund condition. The overall survival rate was approximately 65%. The survival rate by dose groups is shown in the following tabulation (control groups combined).

Diet Level ppm	% Males Surviving	% Females Surviving
0	70	60
10	46	80
50	76	70
250	67	82
1250	44	46
Totals	63	67

The percentage of male survivors in the 10, 250 and 1250 ppm dose groups was less than that of controls. However, only the low and high dose groups were found to be statistically significantly lower than control values (Chi square; $p < 0.05$). Among the females only the 1250 ppm dose group has a greater mortality than controls, but this difference was not significant by the Chi square test. It is noted here for the purpose of clarity that if the low survival rate at 10 ppm in males was truly dose-responsive then all progressively higher doses would have produced a correspondingly increased mortality. This, however, was not seen and therefore it was reasonably concluded that only the highest dose produced a compound related mortality. Therefore, it can be said that except for the highest dose in each sex group no clear dose related pattern was seen.

Time to Death: The cumulative mortality (including moribund animals sacrificed) for males in all dose groups was comparable to the control groups at 60 weeks (i.e. 15 months). A cluster of deaths was noted in the high dose group at 17 and 22.5 months as well as the low dosed group at 22.5 months. Approximately five to six weeks prior to terminal sacrifice (25 months) survival between controls and the intermediate doses was comparable. This was not true however of the low and the high dosed groups where the survival was significantly lower.

The cumulative mortality (including moribund animals sacrificed) for the females, in all dose groups, was generally uniform when compared to control groups, for all time periods, with the exception of females in the high dose group. The survival for the high dosed females decreased sharply between the 61st and 70th weeks (15-17 months) when a cluster of 14 females died. However, only three additional animals died, beyond this point in time, prior to sacrifice.

It can therefore be reasonably concluded, in the opinion of this Toxicology Branch reviewer that an accelerated death rate was not a complicating factor in the analysis of data for either male or female mice.

Tissue Masses: Tables D and E of Volume 1 (Accession No. 241206) respectively indicate the total number of masses (including multiple masses) per animal group per category of classification (i.e. mass; small, medium or large mass; papilloma)as well as the number of animals with at least one mass. It is not clear in this particular instance whether the controls were not pooled or pooled, but whichever the casemay be:

1. *with the former (not pooled), the values for the control group either far exceed the number of tissue masses found in compound dosed groups or far exceed the number of animals with one tissue mass, or*

2. *with the latter ("pooled controls") if this Toxicology Branch reviewer arbitrarily halves the values reported for "pooled controls" the values attained are comparable to those for compound treated groups.*

It can therefore be concluded, in the opinion of this reviewer, that based upon the number of masses, size of the masses, and the numerical distribution of masses within dose groups and between dose groups, the masses per se are not an issue per se, but rather that the types of masses are the issue, and their number.

The distinction between "tissue masses" and the types of masses that are presented within the data is purposely made to avoid any equation or confusion of the term "tissue masses" with the concept of oncogenicity. Many of the masses that were observed during the lifetime of the animal were categorized as suppurative, - nephritis, - hepatitis, - pneumonia, or abscesses, upon necropsy and subsequent histopathological examination.

Tumor types: Males: The only non-neoplastic pathology induced by dietary administration of Pydrin, in male mice, was multifocal granulomata. Pydrin, at 1250 ppm, induced multifocal granulomata in mesenteric lymph nodes, other visceral and peripheral lymph nodes, liver and spleen. Less severe granulomatous lesions were present in mesenteric lymph nodes of male mice administered 50 and 250 ppm Pydrin.

The dietary administration of Pydrin also did not result in statistically significant differences in the number or type of neoplasms, when compared to the incidence of neoplasms in male mice fed the untreated diet concurrently.

Tumor types: Females: The dietary administration of Pydrin to female mice did not result in statistically significant differences in number or type of neoplasms, when compared to the incidence of neoplasms in mice fed untreated diet concurrently.

Multifocal granulomata, in female mice, was the only non-neoplastic pathology induced by Pydrin administration. This lesion was present in mesenteric lymph nodes, liver and spleen at dosage levels of 250 and 1250 ppm. This lesion was not observed at the lower doses of 10 and 50 ppm.

Granulomas: Mesenteric lymph nodes: Multifocal granulomatous lymphadenitis was observed in mesenteric lymph nodes of female mice fed 250 or 1250 ppm of Pydrin and of male mice fed 50, 250 or 1250 ppm Pydrin. The incidence and severity of multifocal granulomata were dose related. Granulomata were observed rarely in mesenteric lymph nodes of female mice fed 50 ppm Pydrin and mice of either sex fed 10 ppm Pydrin or untreated control diet.

Liver: Small multifocal granulomata were identified in the livers of almost all female mice fed 250 and 1250 ppm Pydrin and males fed 1250 ppm Pydrin. The severity of these lesions was dose related in these groups. No treatment related lesions were observed in the livers of mice fed untreated control diet or diet with dosage levels of 10 or 50 ppm.

Spleen: Small pigmented granulomata were present in the spleenic white pulp of almost all female mice fed 250 and 1250 ppm Pydrin and male mice fed 1250 ppm. The severity of of lesions was dose related. No treatment-related lesions were identified in the spleens of mice fed untreated control diet or diet with dosage levels of 10 or 50 ppm Pydrin.

Other Visceral/Peripheral Lymph Nodes: The following nodes were not routinely preserved for microscopy although several were frequently presented for histologic examination. They were submaxillary, inguinal and anterior mediastinal nodes available with salivary gland, mammary glands and thymus respectively. In females granulomata were present in these lymph nodes but lightly pigmented syncytia were observed in

nodal medullary cords in 250 and 1250 ppm groups. This feature, which was frequently identified in females of the 1250 ppm Pydrin group, was present rarely in nodes of female mice fed untreated control diet or diet with dosage levels of 10 or 50 ppm Pydrin. In males, multifocal granulomata, similar to those in mesenteric lymph nodes were present in other visceral and peripheral lymph nodes (apparently at least in dermal lymph node and madibular lymph node; other visceral lymph nodes not determinable by this reviewer but also apparently not that important considering the information available on the reported lymph nodes) in about half of the male mice in the 250 and 1250 ppm Pydrin treated groups. Such lesions were not present in male mice fed untreated control diet or diet with dosage levels of 10 or 50 ppm Pydrin.

Body Weight Data: Males: There were no consistently significant statistical differences between pooled controls and animals fed 10, 50, and 250 ppm Pydrin in the diet. However, in the high dose group (1250 ppm) statistically significant differences were observed ($p < 0.05$; Dunnett's "t-test") for lowered body weights when compared to pooled controls. The reader should also note here that while there was no statistically significant lowered body weights for the 10 ppm group, it was the same group reported earlier which suffered a significant mortality. As one would normally expect a decreased body weight, indicative of compound toxicity, prior to death in these kinds of experiments, this result may also be used as an arguement against the 10 ppm dose level as being dose related for mortality.

Females: No consistently statistically significant differences were noted with respect to decreased body weight for animals receiving 10 and 50 ppm Pydrin in the diet when compared to pooled controls. No consistently significant lowered body weights were noted between treated females and pooled controls for the first 52 weeks of the study at the 250 ppm dose level. However, statistically significant decreases in body weight, which were consistent, were recorded between weeks 60 thru 92 (inclusive) between treated at the 250 ppm dose level and pooled controls. Females receiving 1250 ppm showed consistent and significant decreases in body weight for the duration of the feeding.

The above statistical comparisons were conducted using the Dunnett's "t-test" at a significance level of $p < 0.05$.

Food Consumption: There appears to be no consistent trend and a statistical significance is lacking between treated and control groups.

Hematology: It was reported that all groups were comparable to controls with respect to the various parameters examined. The elevated total leucocyte count in the 10 ppm females was the only statistically significant difference and was considered fortuitous. Examination of the data by this Toxicology Branch reviewer finds no fault with the conclusions as reported.

Clinical Chemistry: Males: The comparison of treated groups to pooled control values for blood urea nitrogen (BUN) and blood glucose (GLCS) revealed neither trends nor significant differences by Dunnett's "t-test" at the 0.05 level of probability. Values for serum alkaline phosphatase (SAP) were comparable between all the animal groups on the test. Values for serum glutamate oxalate transaminase and albumin were comaparable between groups fed 10, 50 and 250 ppm and pooled controls by Dunnett's "t-test" at the $p < 0.05$ level. However, animals fed 1250 ppm showed a statistically significant increase in SGOT levels and a statistically significant decrease in albumin levels.

Review of histopathological data and the clinical chemistry data seemed to indicate that the increased SGOT levels and decreased albumin levels for animals fed 1250 ppm might be ascribed to some degree to the moderate degree of multifocal purulogranulomatous hepatitis as well as to the various degree of severity of multifocal granulomata observed in the lymph nodes, and spleen.

These histopathologic conditions were also observed at the lower dose levels but to a much lesser extent. Review of the heart and kidney data did not appear to implicate these organs as contributory to the increased levels of SGOT or decreased levels of albumin at 1250 ppm.

Females: The comparison of treated groups to pooled control values for BUN and GLCS revealed neither trends nor meaningful statistically significant differences by Dunnett's "t-test" at the 0.05 level of probability. Values for SAP were comparable for all the animals on test. Values for SGOT and albumin were not statistically significant at any dose level. However, a marked increase in SGOT level was noted at 1250 ppm and a sharp decrease in albumin level was also noted at 1250 ppm. The data for the heart and kidney did not appear to implicate these organs as contributory to these effects. However, as in the males, multifocal purulogranulomatous hepatitis and multifocal granulomata of the lymph nodes and spleen may be responsible in whole or part for these values.

Organ Weight: Males: Only kidneys at 1250 ppm showed a statistically significant decrease on an absolute basis when compared to controls. However, body weights were also statistically significantly lower for the same time period. The organ to body weight ratio for males showed no differences for kidney. However, a statistically significant increase in brain weight to body weight ratio was recorded. The significance of this increase was not immediately known.

Females: Absolute organ weights were not lower on a statistical basis for treated animals when compared to controls. The body weight for the high dose group was however statistically significantly lower than controls by Dunnett's "t-test" ($p < 0.05$). A statistically significant increase in the organ to body weight ratio was recorded for the kidneys at the high dose level at the 0.05 level using Dunnett's "t-test".

No strong trends or dose-response curves appear evident for either sex in both categories of measurement.

Summary and Conclusion: Dietary administration of SD 43775 to male and female mice for two years at concentrations of 10, 50, 250 and 1250 ppm caused clearly increased mortality in both sexes only at the highest feeding level, in comparison to untreated controls. Mortality was not dose-related at the lower diet concentrations (the 10 ppm males had nearly the mortality percentage as was found in the highest dose).

Mean body weights were statistically significantly reduced, compared to controls in the 1250 ppm mice (both sexes). While these reductions were fairly consistent throughout the study, body weights of mice in the other groups showed no clear pattern with dose or time, except that the 250 ppm females began to lag and were statistically significantly lower from controls from about the 60th week on to the time of terminal kill.

Tissue masses observed grossly throughout the study were not dose related.

The only clinical chemistry changes observed (serum albumin reduction and SGOT elevation) were in the 1250 ppm group. Hematology findings were not exceptional.

Organ weights and organ-body weight ratios gave no real indications of a dose-relationship.

Dietary administration of Pydrin to male and female mice for two (2) years did not result in statistically significant differences in the number or type of neoplasms, when compared to the incidence of neoplasms in mice fed untreated diet concurrently.

The only non-neoplastic pathology induced by dietary administration of Pydrin was multifocal granulomata. In female mice, this lesion was present in mesenteric lymph nodes, liver, and spleen at dosage levels of 250 and 1250 ppm Pydrin. In male mice; 1250 ppm Pydrin induced multifocal granulomata in mesenteric lymph nodes, other lymph nodes, liver and spleen. Less severe granulomatous lesions were present in mesenteric lymph nodes of male mice administered 50 and 250 ppm Pydrin.

NOEL: Males: 10 - 50 ppm
Female: 50 - 250 ppm

LEL: Males: 50 ppm, multifocal granulomatous lymphadenitis of the mesenteric lymph nodes.

Females: 250 ppm, multifocal granulomatous lymphadenitis (mesenteric lymph node); multifocal granuloma of the liver; small pigmented granulomata in the splenic white pulp. Statistically lower mean body weights after 60 weeks.

HEL: Males: 1250 ppm, multifocal granulomatous lymphadenitis (mesenteric); multifocal granuloma of the liver; small pigmented granulomata were present in the splenic white pulp. Increased mortality. Statistically significant reduction in body weight. Increased SGOT. Decreased serum albumin. Increased brain/body weight ratio.

Female: 1250 ppm, multifocal granulomatous lymphadenitis (mesenteric); multifocal granulomata of the liver; small pigmented granulomata were present in the splenic white pulp. Increased mortality. Statistically significant reduction in body weight. Increased SGOT. Decreased serum albumin. Statistically significant kidney/body weight ratio increase.

Classification: Core-Guidelines

Addendum: Reference: PP 7F2013. Review of Reto Engler dated July 21, 1978.

Pydrin was administered in the diet to d d y strain mice for an 18 month period. Doses administered were 100, 300, 1000 and 3000 ppm.

The results and conclusions of the study using d d y strain mice, which was reviewed by Reto Engler, of the Toxicology Branch are generally parallel to and confirm the results and conclusions reported in the study, using B6C3F1 strain mice, which was reviewed by Albin Kocialski.

The results and conclusions of the two studies, with regard to granulomas, effect levels and oncogenicity were:

1. *Oncogenicity is not indicated,*
2. *granulomatous changes in lymph nodes, spleen and liver were present and related to dose level,*
3. *the 100 ppm dose level was the lowest effect level (LEL) for effects in d d y strain mice and a No-Observable-Effect-Level was not observed,*
4. *the no-observable-effect-level for B6C3F1 mice was between 10 and 50 ppm.*

Reviewer's Questions:

1. *Portions of liver and mesenteric lymph nodes from each of five mice per sex from the controls and high dose groups, that were killed at termination were preserved in glutaraldehyde, for special processing, presumably for examination under the electron microscope. The Toxicology Branch would like to know the purpose of the examination the results and the interpretation given to the electron microscopy examination.*

2. *What statistical method was used to analyze for the statistical significance of tumors?*

Pages 39-52 - * Claimed confidential by submitter*