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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 Beliles, 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.

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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 Psuedomonas 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% Techmical

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 caseмay 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 frequantly 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 lymp. 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 splaenic 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, presumally 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?*

PYDRIN

Page_____ is not included in this copy.

Pages 13 through 14 are not included.

The material not included contains the following type of information:

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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.
Toxicology Branch/HED (TS-769)

NBK 3/2/81

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.

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Fenvalerate: 2-Year Feeding/Carcinogenicity Study in Mice
Shell Chemical Company. MRID No. 00071949. HED Doc. No. 009003

009003 (4-3-81, 52 pgs) on last page of attached