

The "Bressler Report"

(Mammary tumor "Adenocarcinoma" found in 1972 animal study pp6,67,70)

(Note: This is the text of an FDA report on Searle)

EIR 4/25/77 to 8/4/77
JSA/DME/JT/LF

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SUMMARY OF FINDINGS

Authentication of this study was performed primarily by comparing available raw data with the submission to FDA. This was a problem, at times, due to the lack of some data and difficulty in locating other material. The majority of material relating to Aspartame was already under FDA seal at Searle. However, during this investigation we discovered various documents and notebooks that were not.

In some cases original data could be recorded in several areas, making it difficult, and sometimes impossible to determine which was actually the original. This was a particular problem in dealing with dates of deaths, as some conflicted on the "source" documents. Many of the responsible individuals involved with the study, including stability testing of DKP, are no longer employed by Searle. Dr. K.S. Rao, Study Monitor, the only individual who could have possibly answered some questions, had left Searle. He was contacted, but permission for an interview was refused by his attorney. Due to the absence of various individuals it was not always possible to accurately determine methods used in some analyses and operations carried out in conducting this study. In a number of areas, including chemistry, statistics, diet preparation and feeding, it was necessary to use assumptions, or information supplied by current employees who were not involved with the study.

At the beginning of this investigation, Mr. James R. Phelps, Vice-President and General Counsel for G.D. Searle & Co., advised us that an attorney and scientific coordinator would have to be present at all times to protect their interest in the data. This did not present any insurmountable problems, but on several occasions an attorney would question our request for data, stating that it was not relevant for authentication. At no time did we make any statement to the effect that our goal was to authenticate the study. Two memos were discovered dealing with reaction of animals to the diet. This was a significant factor in the study. Permission to copy them was initially refused, but finally granted after Searle was contacted by FDA General Counsel. We were not allowed to make xerox copies of any documents for about two and one-half weeks, due to Searle's concern over confidentiality. This was eventually reconciled between Searle and FDA General Counsel.

The major discrepancies concerning Study PD 988S73, SC-19192: 115 Week Oral Tumorigenicity Study in the Rat, are as follows:

A. Design and Conduct of Study

- 1) Control and treated animals were randomly distributed on the same rack. (See diagram of housing group attached as exhibit 7.)
- 2) No ear clips or other methods of uniquely identifying each animal were used. Identification consisted of two types of cards attached to the front of each cage.
- 3) Compound inventory cards were deficient in that only one of 18 such cards stated the purpose (study 988S73) for withdrawing the compound from inventory. Three of the cards did not include the date withdrawn, amount withdrawn, or signature of requestor. Therefore it was impossible to Reconcile the amount withdrawn and the amount used. (See exhibit #28.)
- 4) Food jars were not individually identified, yet all the filled jars for a given housing group (control, low, mid, and high dose) were placed on a mobile cart, which was wheeled to the housing rack. The position of the jar (in rows) on the cart was the only means of identifying the proper dose level. The arrangement of the food cups on the cart is shown in exhibit #8.
- 5) A total of 79 "observations for drug effects" records were not signed or initialed.
- 6) Observation records indicated that animal A23LM was alive at week 88, dead from week 92 through week 104, alive at week 108, and dead at week 112.
- 7) Records indicated that at the scheduled 104 week bleeding, animal E2CM was substituted for animal A11CM. Records also indicated that animal A11CM was alive on this date and therefore should have been bled as scheduled.
- 8) Records indicated that penicillin was administered to four rats beginning on May 16, 1973, and continuing daily through May 28, 1973. This third occurrence of infections disease and penicillin administration was not reported in the submission to FDA.

- 9) In many cases the actual number of tissues embedded was less than the 24 (control and high dose) or 19 (low and mid dose) specified in the final histology lab protocol dated 1/21/74.
- 10) Ophthalmoscopic examination records were present for animals H26MF and J29CM, yet the findings were not reported in the submission to FDA. Two other discrepancies of this type were noted.
- 11) Records indicate that a tissue mass measuring 1.5 x 1.0 cm was excised from animal B3HF on 2/12/72, and that a "skin incision over mass" was performed on animals C22LM and G25LM on Feb. 10, 1972.

B. Stability and Homogeneity of DKP in Diet Mixtures

- 1) There were no batch records to show the quantities of DKP and basal diet weighted, type of mixer used, mixing time, dates, or names of individuals performing the weighing and blending operations.
- 2) There was no evidence that any tests had been done to determine the blending characteristics of the mixer, or to validate the mixing time.
- 3) No homogeneity tests were performed on any batches of diet used in the study, and to stability study assay reports (A7738 and A7739) indicated that samples were not homogeneous. (See exhibit #29.)
- 4) A stability study was conducted with DKP in 1972. However, the 115 week rat stud employed Basal Diet from week 2 to its conclusion, and to stability studies had been conducted with Basal Diet.
- 5) Methods of assay for DKP in the diet were deficient in that: The titration method was discontinued after 1 week of the stability study. Some of the TLC photographs showed no DKP reference standards and photographs also showed that there was something in the basal diet itself producing a spot on the TLC plate which had an Rf value corresponding to DKP. Only one solvent system was used for development of the TLC Plates. Some of the chromatograms showed poor separation.

- 6) No reserve samples of any of the lots of DKP used in this study were retained by Searle.
- 7) Three different sets of specifications for DKP were found, and Searle could not determine with any degree of certainty which of the three were applicable to the 7 lots of DKP used in the study.
- 8) The analytical records for DKP lots IR through 5R refer to reference standard IR #3701. None of the three sets of DKP specifications lists reference #3701. No data was made available as to dates, methods of preparation and authentication of DKP reference standards.
- 9) Analytical records A-9129 for DKP lot 5R showed an assay of 1000%. Examination of laboratory notebooks showed that eleven (11) samples had been analyzed from this lot, and the analytical record only reflected an average of the last three of these. The other assays (not reported) ranged from 87.93% to 114.83%.

C. Dosage, Body Weight and Food Consumption

- 1) Examination of the raw data sheets revealed the following discrepancies:
 - a. Empty feed cup weights were missing for the D housing group at the 12th week, in the raw data sheets. (See exhibit #75.)
 - b. In several instances, the dietary concentration shown on the weight sheets did not agree with the concentration listed for the same level in the other housing groups. (For example; C group Males, mid & high levels for week 13,; A group Males, high levels for week 99.)
- 2) Comparison of the Searle submission and the independent FDA analysis of the raw body weight and food consumption data revealed the following discrepancies:
 - a. We found a total of 15 differences of 1 gram or more in the average body weight and of 0.1 percentage points or more in weight gain. (See table 1.)

- b. We found approximately 82 discrepancies of one gram or more in the food intake when expressed in grams/day. (See table 2.)
 - c. We found approximately 40 errors of 5 or more grams in food intake when expressed in grams/kg./day. (See table 2.)
 - d. Most of our dosage calculations differed from Searle's dosage calculations by 10 or more mg., when the dosage is expressed as mg/kg/day. (See table 2.)
- D. Gross and Microscopic Pathology
- 1) 98 of the 196 animals that died during the study were fixed in toto and autopsied at some later date, in some cases more than one year later.
 - 2) A total of 20 animals were excluded from the study due to excessive autolysis. Of these, 17 had been fixed in toto and autopsied at a later date.
 - 3) Records indicated that animal F6HF, a high dose female, was found dead at 787 days of treatment and the gross pathology sheet reported a tissue mass measuring 5.0 X 4.5 X 2.5 cm. The submission to FDA reported no tissue mass and the animal was excluded from the study due to marked autolysis.
 - 4) Records for approximately 30 animals showed substantial differences between gross observations on pathology sheets, when compared with the gross observations on pathology sheets submitted to FDA. A detailed description of 10 of these is included in the report. Copies of all the gross pathology sheets, and the pathology summaries submitted to FDA are attached as exhibits.
 - 5) Dr. Charles H. Frith, D.V.M., Ph.D., Directory, Pathology Services, NCTR, examined slides for a total of 150 animals, or about 42 percent of the animals on study. He noted the following discrepancies:
 - a. The reporting of a mass (by Searle) as missing which was actually present (animal M1LF.)

- b. The finding of a **polyp of the uterus** which was not diagnosed by Searle (animal K9MF). The finding of this additional uterine polyp by Dr. Frith increases the incidence in the midi dose to 5 of 34. **(15 percent.)**
 - c. The finding of **ovarian neoplasms in animals H19CF, H19C, and H7HF**, and the finding of diffuse hyperplasia in animal D29CF, which were not diagnosed by Searle.
 - d. The finding of additional inconsistencies in 21 animals.
- 6) No microscopic worksheets or other "raw data" relating to microscopic pathology could be found for this study.
 - 7) **A mammary tumor found in animal F27CF** was described as a papillary cystadenoma on the pathology summary sheet, (page 105, Vol. II of the submission) and as an **adenocarcinoma** on summary table 12 (p. 95, Vol. I of the submission).
 - 8) In several instances the histopathology technician made notes at the bottom of the gross pathology sheet to indicate that certain organs were not present in the bottle of fixative (and were therefore not available for sectioning). Yet, in three of these instances (animals A4CM, K23CF, and J3CM) a diagnosis appears in the submission to FDA.

E. Organ Weights

- 1) Organ weights were entered on the gross pathology sheets at the time of autopsy. We compared all of the individual organ weights on appendix table 5 in the submission to FDA (Vol. 1, pgs. 222-226) with the original data on the gross pathology sheets. A total of eleven (11) errors were noted in transcribing the raw data from the pathology sheets to the tables in the submission to FDA.

F. Survival

- 1. We were unable to determine the exact method used by Searle in constructing the survival table in the submission to FDA. We constructed a survival table using the body/feeder weight Teletype sheets. A Life Table Analysis was constructed from our survival table by Dennis Wilson, FDA Department of Mathematics. The female control population differed from the high level population (p 0.05) and the mail control population differed from the mid and high level population (p 0.05). In all cases the differences are due to higher mortality in the controls.

G. Clinical Laboratory Procedures

1. Laboratory records of one sort or another for all assays reported in the submission were obtained. In some cases data sheets were noted with results of assays carried out at treatment days not indicated in the protocol or protocol amendment. For example, serum cholesterol determinations were done at days 796 and 798 (terminal bleeding) but not included in the submission to FDA. Because the submission to FDA (Vol. 1 p. 286) reported a significant decrease in serum cholesterol that was more perceptible towards the end of the study, and may have been related to compound administration, the omitted data is of some importance.
2. No data was seen for two assays (serum insulin and serum ornithine carbamyl transferase) which were called for in an amendment to the protocol.
3. Original data was not always available for authentication of results or examination of procedures for conversion of raw data into the calculated values submitted to FDA.
4. Data pages for clinical chemistry and urinalysis were initialed by a technician who transcribed data but apparently was not directly involved in the assays indicated. He stated in an interview that Dr. Rao told him to initial the data sheets.
5. The methodology as referenced in the submission to FDA is incomplete and not always an accurate reflection of the methodology actually used in the study. The fact that more than one method was sometimes used for a particular assay during different times of the study was not indicated in the submission to FDA.
6. A total of 21 disparities between individual clinical laboratory analysis values appearing in the submission Volume I and those values appearing in data sheets and/or laboratory notebooks were found.
7. A total of 49 disparities were noted between statistical computations reported by Searle in the submission and those calculated by FDA. The disparities are constituted by the values for 6 means, 23 standard errors, and 20 significant differences (as measured by T tests).
8. Some of the data sheets for urinalysis had erroneously labeled the phenylketones test values as "phenylalanine".

PURPOSE OF INVESTIGATION

Assignment memo dated may 16, 1977 from Donald Healton, Acting Director of Regional Operations, confirmed an earlier oral assignment to Chicago District for a directed inspection of certain non-clinical studies submitted to FDA in support of a food additive petition for the sweetener aspartame.

The investigation began on 4/25/77, and encompassed the authentication of all data, both raw and summary, relating to the studies jointly chosen for review by Bureau of Foods and EDRO. Two studies actually done at G.D. Searle were selected for initial coverage, and a decision to expand the investigation to a third study was made at a later date.

Following are the titles of the three studies selected for review:

- 1.) E-5 (P.T. #851S70), Evaluation of the Embryotoxic and Teatogenic Potential in the Rat, conducted with SC-18862 (aspartame).
- 2.) E-89 (PT #1218S75), An evaluation of the Embryotoxic and Tetatogenic Potential in the Mouse, conducted with SC-18862 (aspartame).
- 3.) E-77/78 (PT #988S73), 115 Week Oral Tumorigenicity Study in the Rat, conducted with SC-19192 (diketopiperazine).

This report is concerned only with study E-77/78. The report of E-5 and E-89 was submitted separately.

HISTORY OF BUSINESS

G. D. Searle & Co. provides a wide range of health care products and services on a worldwide basis. Its business is divided among three principal areas: pharmaceuticals, medical instruments and optical products, and hospital and laboratory products. The firm's corporate offices are located in Skokie, Illinois, with various branches and facilities throughout the world.

Effective June 1, 1977, Donald H. Rumsfeld assumed duties as President and Chief Executive Officer. Mr. Daniel C. Searle, formerly Chief Executive Officer is now Chairman of the Board, while William L. Searle and Wesley M. Dixon, former Chairman and President respectively, are now Vice-Chairmen.

Effective March 1, 1977, the firm underwent a major realignment, shifting to a managerial system based on product lines. This resulted in the establishment of four main product-line groups, which are: Pharmaceutical/Consumer Products, Diagnostics, Hospital Supplies and Optical Products. Each group is headed by a President who will report to Searle's Executive Vice-President for Operations, Dr. James A. Buzard. A copy of the G. D. Searle & Co. annual report for 1976 which is attached as Exhibit #1 further expands on the firm's operations and lists Corporate Officers.

Mr. O. B. Parrish is President of the Pharmaceutical/Consumer Products Group and also a Corporate Vice-President. An organizational chart for this group is attached as Exhibit #2. Mr. Guy Labrosse is now Group Executive Vice-President for U. S. Commercial Pharmaceutical Operations. In the U. S., this is known as Searle Laboratories. The facility at 4901 Searle Parkway, Skokie, Illinois is a part of the U. S. Operations, e. g. Searle Laboratories, yet houses the majority of the Research and Development Group.

Worldwide Pharmaceutical Research and Development is also a part of the Pharmaceutical/Consumer Products Group, but not of Searle Laboratories. The Research and Development OF aspartame is a function of this group. Copies of organizational charts for this group are attached as Exhibit #3. Dr. Robert A. Moe recently resigned and his position is temporarily being filled by George V. O'Bleness, Corporate Vice-President for Compliance and Administration.

Commercial aspects of Aspartame are being handled by an "aspartame Division", under the direction of Elwood H. Ensor, Corporate Vice-President. There is no longer a division entitled "New Ventures".

PERSONS INTERVIEWED

Credentials were shown and a written Notice of Inspection was issued to Dr. William M. Merino, Director, Domestic Pharmaceutical Products, Regulatory Affairs Department on April 25, 1977. The following Searle personnel were present at the initial meeting on 4-25-1977.

Robert A. Moe, PhD. - Executive Vice-President
George Clay, PhD. - Group Leader, CNS Pharmacology
Robert Bost, PhD. - Director of Food Products,
Regulatory Affairs
Holly Ru Probst - Director, Corporation Information
Management Group.
Dave Britton - Director Corporation Information
Department
William Merino, PhD. - Director, Domestic Pharmaceutical
Products
Richard Viktora - Attorney
James Phelps - Vice-President, General Counsel
Elwood H. Ensor, PhD. - Vice-President
Paul Klimstra, PhD. - Vice-President Pre-Clinical
Research and Development
Roger Thies - Attorney

During the course of our investigation one or more of the following Searle personnel were present in the Conference Room which we used for our data review.

Richard Viktora - Attorney
Roger Thies - Attorney
George Clay, PhD. - Group Leader, CNS Pharmacology
Robert Bost, PhD. - Director of Food Products
Regulatory affairs
Don Cook, PhD. - Associate Director, Department of
Bio Research
Dick Aspinol, PhD. - I. I. D. Group Leader
Bill Jenkins, PhD. - Director, Product Affairs
Fred McIlreath, PhD. - Director, Regulatory Affairs
Paul Landefeld, - Attorney

Most of the time one attorney (R. Viktora or R. Thies) and one scientist were present. During our initial meeting with Searle personnel, James Phelps stated that a Searle monitor must be with us at all times during our data review in order to "protect the data".

During the course of our investigation, various individuals were interviewed in an attempt to obtain all available raw data and reconstruct the manner in which the study was conducted, as accurately as possible. Since many employees involved in the study or support areas are no longer employed at Searle, others were interviewed who had general knowledge of such parameters as statistics and chemistry.

Significant interviews are attached as Exhibits, as referenced.
Individuals interviewed were as follows:

1. Donna Helms - Administrative Assistant to Dr. McConnell on 5-18-77, 6-30-77 and 7/1/77 (Exh. #46).
2. Judith Beauchamp - Hematology Lab Supervisor on 6-2-77 (Exh. #47).
3. Barbara Bickford (Nee Ross) - Technician, Department of Analytical Research on 6-1-77 and 6-2-77 (Exh. #48).
4. Clifford J. Suel - Supervisor, Department of Analytical Research and Development on 6-2-77 (Exh. #49).
5. Bartolome R. Tangonan - Research Technician, Pathology Toxicology Department on 6-1-77 (Exh. #50).
6. Tony Martinez - Research Assistant and Toxicology Lab Supervisor on 5-19-77, 6-3-77, 7-7-77, 7-20-77 and 8-2-77 (Exh. #51).
7. Ted Reichert - Supervisory Systems Analyst on 5-24-77 (Exh. #52).
8. Phil Polli - Systems Analyst on 5-24-77 (Exh. #53).
9. Judith Schmal - Clinical Chemistry Section Supervisor on 6-2-77 and 6-7-77 (Exh. #54).
10. Jane Drury - Analytical Chemist, Bioanalytical Dept. 6-7-77.
11. Alan Mitchell - Teratologist on 7-20-77 (Exh. #56).
12. Raymond G. Schroeder - Former Searle Teratologist on 7-18-77 (Exh. #57).
13. Dr. Rudolph Stejskal - Pathologist on 6-23-77.
14. Patricia Erdenberger - Research Assistant and Histopathology Lab Supervisor on various dates (Exh. #58).

Dr. Robert McConnell, Pathology-Toxicology Advisor at the time of this study, was not directly involved with daily procedures. He is no longer employed at Searle.

An attempt was made to interview Dr. K. S. Rao, Monitor of Study P. T. #988S73 on 7-25-77. We were referred to Dr. Rao's attorney, who refused permission for an interview (see Jerome Bressler's memo dated 7-27-77, Exh. #33).

PURPOSE OF STUDY PT 988S73 (E-77/78)

SC-19192: 115 Week Oral Tumorigenicity Study in the Rat

According to the submission to FDA, this study was intended to evaluate the safety and tumorigenic potential of SC-19192, diketopiperazine (5-benzyl-3, 6-dioxo-2-piperazine-acetic acid), which is a conversion product of aspartame, and to induce and define such adverse effects as might occur only at prodigious multiples of the estimated daily human intake. The commercial grade of aspartame (SC-18862) may contain up to 2 percent of the conversion product (DKP), according to Searle's specifications.

DATES

Study e-77/78 (PT #988S73) was initiated on November 8, 1971. The study was to be terminated at 104 weeks, but was extended to 115 weeks. The reason for extending the study was stated as follows in protocol amendment #3 dated September 6, 1973: "it was decided to extend or continue the study until the mortality of either sex reduced the control group to 20 animals per sex, provided the survival in the treated groups is not less than 10 animals/sex/treated group prior to that period. This approach is consistent with current FDA desires." A copy of the study protocol is attached as exhibit #11.

Initiation of treatment was staggered over a two week period as follows:

HOUSING GROUP	DATE PLACED ON STUDY	SCHEDULED SACRIFICE	DAYS ON STUDY
A - Male	11/8/71	1/21/74	805
B - Female	11/9/71	1/22/74	805
C - Male	11/9/71	1/22/74	805
D - Female	11/10/71	1/23/74	805
E - Male	11/11/71	1/24/74	805
F - Female	11/12/71	1/25/74	805
G - Male	11/15/71	1/28/74	805
H - Female	11/16/71	1/29/74	805
J - Male	11/17/71	1/30/74	805
K - Female	11/17/71	1/30/74	805
L - Male	11/18/71	1/31/74	805
M - Female	11/19/71	2/1/74	805

PROTOCOL AND AMENDMENTS

A copy of the protocol for this study was obtained and is attached to this report (See Exhibit #11). The protocol includes 4 amendments which are dated Aug 20, 1973, (amendments #1 and 2), Sept. 6, 1973 and Jan 9, 1974.

Amendment #1 dated Aug 20, 1973 specified 4 additional clinical chemistry laboratory measurements: 1.) serum insulin, 2.) serum ornithine carbamyl transferase, 3.) serum protein electrophoresis, 4.) serum total protein.

Two of the above assays (serum insulin, and serum ornithine carbamyl transferase) were apparently not done, because no data for these two parameters was submitted to FDA, and we could find no raw data or other evidence that they were done.

Amendment #2 dated Aug 20, 1973, specified 8 coronal sections of brain to be examined microscopically, and also described the procedure for sectioning the urinary bladder. Four transverse sections from each urinary bladder were to be examined microscopically.

Amendment #3 dated Sept. 6, 1973 extended the study until it reached a point where mortality reduced the control group to 20 animals per sex, provided survival of treated groups was not less than 10 per sex per group. (This represented a survival of approximately 30%).

Amendment #4 dated Jan 9, 1974 added serum cholesterol to the clinical chemistry measurements to be made at terminal sacrifice, and terminated the study after 114 weeks of treatment. Terminal sacrifice was to begin on 1-24-74 and continue through 2-1-74.

Our examination of the original data showed that serum cholesterol determinations were done at day 796 and 798 (terminal bleeding) as specified in the above amendment, but the data was not included in the submission to FDA. The submission to FDA (Vol. 1 p. 286) reported a significant decrease in serum cholesterol that was more perceptible towards the end of the study, and may have been related to compound administration. Therefore, the omitted data may have been important.

Serum cholesterol determinations were also done at day 546 (78 weeks) and not reported in the submission to FDA.

The protocol for Clinical Chemistry procedures specified that BUN determinations were to be done at 78 weeks (546 days). The submission to FDA contained no BUN data for day 546, but our review of the raw data indicated that BUN's had been done at day 546. Some BUN's were also done at day 735 (105 weeks) and not reported in the submission to FDA, but this data was not complete for all animals.

Attached to the protocol is a memo dated Oct. 31, 1972 which describes an acute infection spreading in the rat colony, and the administration of penicillin to combat the infection, and a memo dated May 8, 1973 listing scheduled dates to be added to Body and Feeder Weights of housing groups A & B.

The final Histology Lab Protocol, dated 1-21-74, specifies 24 organs to be embedded for control and high dose animals, and 19 organs to be embedded for low and mid dose groups. The organs which were to be embedded for the control and high dose groups but to be omitted in the low and mid dose groups include: lymph node, nerve, bone, eye, and salivary glands.

Pathology sheets (blank forms) to be used at terminal sacrifice were reproduced (xeroxed) with check marks, time (death to tissue fix), fixative, study, and project number already entered. Twenty-seven (27) organs were checked off, to be embedded. However, as stated above, the control and high dose animals were to have 24 organs embedded, according to the protocol, and the mid and low dose 19. Therefore, all pathology sheets for animals killed by design have incorrectly identified the specific organs and tissues to be embedded.

In addition to the above error, in many cases the actual number of tissues embedded was less than the 24 (control and high dose) or 19 (low and mid dose) specified in the final Histology Lab Protocol dated 1-21-74. Specific figures for numbers of tissues embedded at terminal sacrifice are as follows:

	ACTUAL RANGE	ACTUAL AVERAGE	NUMBER SPECI- FIED IN PRO- TOCOL	NO. OF ANIMALS NOT IN ACCORD WITH PROTOCOL
CONTROLS	10-24	20	24	129 of 144
LOW DOSE	12-23	19	19	19 of 72
MID DOSE	4-24	18	19	28 of 72
HIGH DOSE	9-25	22	24	51 of 72

PERSONNEL AND RESPONSIBILITY

The names of Dr. K.S. Rao, Dr. R. Stejskal, and Dr. R.G. McConnell appear on the final study report, indicating that they are the authors of this report, and were responsible for the study.

Following are the principal person involved with study E-77/78 and their specific areas of responsibility:

- 1.) Dr. Robert G. McConnell - Director, Pathology-Toxicology Laboratory 1970 through 1974. Dr. McConnell functioned as the Path-Tox advisor on study E-77/78. He is no longer employed by Searle.
- 2.) Dr. Suryanarayana K. Rao - Manager, General Toxicology Laboratory, June 1971 until he left Searle in May of 1977. Dr. Rao was the Path-Tox monitor for study E-77/78. In 1971 Dr. Rao monitored 30 studies, in 1972 forty-seven (47) studies, in 1973 twenty-nine (29) studies and in 1974 twenty-five (25) studies.
- 3.) Dr. Rudolf Stejskal - Senior Research Investigator, Pathologist. Dr. Stejskal was responsible for the microscopic findings and accuracy of these findings in the study report of E-77/78. Because Dr. Stejskal joined Searle in July, 1973, he had no input into the pathology protocol. Also, he did not examine all of the slides for this study, but was assisted in that task by Dr. Joseph H. Smith M. D..
- 4.) Dr. Joseph H. Smith, M.D. - Group Leader and Senior Pathologist at Michael Reese Hospital, Chicago, IL., before joining Searle in June of 1973. Dr. Smith examined some of the slides for study E-77/78, and supervised the necropsy laboratory.
- 5.) Tony Martinez - Toxicology Laboratory Supervisor, 1970 through 1973. Mr. Martinez participated in twelve (12) studies in 1971, Seventeen (17) studies in 1972, and thirteen (13) studies in 1973. Mr. Martinez supervised the technicians who worked on the study. He also performed some necropsies.

- 6.) David K.T. Kie, B.S., Research Assistant in Pathology Laboratory. He performed some of the necropsies on E-77/78.
- 7.) Robert Spaet - Research Assistant. He also performed necropsies.
- 8.) Bartolome R. Tangonan - Research Technician II - He was involved with preparation of diet mixtures, daily observations, weighing and feeding animals, etc.
- 9.) Donna K. Helms - Manager, Safety Evaluation, Project Scheduling, Reporting, and Data Storage, Path-Tox Dept. and Administrative Assistant to Dr. McConnell.
- 10.) Patrica Erdenberger - Research Assistant, and Histology lab Supervisor.
- 11.) Dr. Eugene Joseph Youkilis - Senior Research Investigator. He performed the ophthalmoscopic examinations in study E-77/78.
- 12.) Judy A Henderson - August 1972 to present, Research Technician III, Histopathology Dept. She was involved with tissue processing on study E-77/78.
- 13.) Judith R. Schmal - Nov. 1971 to present, Supervisor, Clinical Chemistry Section of Bioanalytical Laboratory.
- 14.) Judith A. Beauchamp - Employed Aug, 1970 to present; Supervisor Hematology laboratory since April 1973.
- 15.) Barbara (Ross) Bickford - Research Technician, Quality Control Department. She performed analyses of DKP diet mixtures for study E-77/78.
- 16.) Clifford J. Seul - Supervisor, Method Development, Stability Evaluation Laboratory. He was Barbara Bickford's supervisor at the time the DKP stability study was performed.
- 17.) Jack Droggt - 1967 to present, Senior Research Assistant, Chemical Development. Mr. Droggt manufactured the 7 lots of DKP used in the study E-77/78.
- 18.) Dr. John E. Dutt - Math-Stat. Dept.
- 19.) John Mellman, Math-Stat Dept.

20.) Fred Hunter, Technician.

Since the Task Force investigation in 1975, there has been a major internal reorganization. The current organization of Worldwide Pharmaceutical Research & Development is attached as Exhibit #3. The only change has been the resignation of Dr. Robert A Moe, Executive Vice-President. Mr. George V. O'Bleness, Corporate Vice-President, is temporarily filling this position.

Organizational charts for Preclinical Research and Development of Products safety Assessment are also attached as Exhibits 4 & 5. There have been no changes in these areas to date.

Worldwide Pharmaceutical Research and Development is responsible for research and development of Aspartame and is a part of the Pharmaceutical/Consumer Product group. The group President is O.B. Parrish, who reports to James A Buzard, Executive Vice-President for Operations, G.D. Searle & Co. The current corporate structure of G.D. Searle & Co. has been discussed under History of Business.

P.T. No. 988S73, 115 Week Oral Tumorigenicity Study in the Rat was conducted between November 10\971 and February 1974. The final FDA submission was dated September 1974. Following is a yearly breakdown of key personnel during this study:

1971

Robert Moe - Director, Biological Research Department.
Robert McConnell - Director, Pathology - Toxicology Section
K.S. Rao (June, 1971) - Manager, Toxicology Section.
Tony Martinez - Toxicology Laboratory Supervisor.

1972

Robert Moe - Director Biological Research Department
(January through April)

F. Saunders - Director, Biological Research Department (May
through December).

Robert McConnell - Director, Pathology - Toxicology Section

K.S. Rao - Manager, Toxicology Section.

Tony Martinez - Toxicology Laboratory Supervisor.

1973 (January to June)

Francis Saunders - Director, Biological Research Department.

Robert McConnell - Director, Pathology-Toxicology Section.

K.S. Rao - Manager, General Toxicology Laboratory.

Tony Martinez - Toxicology Laboratory Supervisor.

1972 (July to December)

Paul Klimstra - Director, Pre-clinical Research & Development
Department.

Robert McConnell - Director, Pathology-Toxicology Section.

K.S. Rao - Manager, General Toxicology Laboratory.

Tony Martinez - Toxicology Laboratory Supervisor.

1974

Paul Klimstra - Director, Pre-clinical Research & Development
Department.

Robert McConnell - Director, Pathology-Toxicology Section.

K.S. Rao - Manager, General Toxicology Laboratory.

D. Semler - Toxicology Laboratory Supervisor.

A more complete listing of personnel in the Department of
Science, from 1971-1975 is attached as Exhibit No. 64.
This includes the Pathology - Toxicology Department and other
ancillary areas.

Curriculum vitae for individuals performing significant func-
tions in the study are attached as Exhibit 12.

MANUFACTURE AND TESTING OF SC-19192

Seven batches of SC-19192 (diketopiperazine) were used in this
study. All batches were manufactured in-house by Searle Chemist
jack Drogt. The lot numbers, analytical numbers, and quantities
are as follows.

Lot Number	Analytical Number	Quantity (After Milling)
1R	6906	
2R	7274	
3R	7273	
4R	7291	This data removed in released report
5R (JDR-5-18A)	9129	
6R (JDR-5-30A)	9805	
7R (JDR-5-30B)	9829	

Bach records covering the manufacture of lots 1R through 5R were reviewed. Batch records for lot 6R and 7R could not be located by Searle personnel. analytical reports for all seven batches were reviewed. Copies of the batch records and analytical records were obtained and are attached to this report, along with copies of pages from Jack Drog't's laboratory notebook, and other laboratory notebooks relating to the analysis of lots 1R through 7R of DKP. (See Exhibits 13-23.)

We obtained copies of three different specification sheets for DKP. (See Exhibits 16-18.) We could not determine with certainty which of the three specifications sheets was in effect at the time that the 7 lots of DKP used in this study were assayed, because only one of the three specification sheets was dated. This resulted in ambiguities for two of the parameters measured: melting point and identity (IR Spectrum). Specification memorandum dated Dec. 4, 1969 listed a melting range of 252-256 degrees C. Another specification sheet (not dated) entitled "Tentative Specification For SC-19192", listed a melting range of 241-246 degrees C. A third specification sheet entitled "Specification for SC-19192, Specification No. C 40606C" (not dated) listed a melting range "at about 243 degrees C."

For identity (IR Spectrum) the first sheet (dated 12/4/69) specified that "The reference standard shall be considered to be TJT-12-32 until something better comes along". The second and third sheets specify that the DKP "Conforms to IR #2358".

No data was made available as to dates, method of preparation and authentication of DKP references standards used.

Searle attorney Roger Thies was contacted about this point Aug. 1, 1977 and said he would attempt to obtain information regarding this point but later registered doubt as to whether anything would be found.

We asked Searle personnel to tell us which of the specification sheets was valid for the DKP used in study E-77/78. We were

told that the third sheet, identified with "No. C4060C", could not have been used since the number corresponded to a date in June, 1974.

It is not clear as to the exact date that the first sheet (dated 12/4/69) was superceded by the second one, identified "tentative specifications for SC-19192" because the second sheet was not dated or numbered. However, Searle attorney Roger Thies told us that their "best guess" was that the sheet marked "tentative specifications for SC-19192" was the one used.

Accordingly, we have used the specifications from the sheet marked "tentative specifications" for the following chart, which compares the specifications with the actual results of analysis.

DKP LOTS

Specifications	1R	2R	3R	4R	5R	6R	7R
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Specifications 1R 2R 3R 4R 5R 6R 7R

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Note... This entire table was expunged from the delivered document, by parties unknown.

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The only discrepancy apparent in the above chart is in the criteria for identity. The specification lists reference standard 1R #2358, while the analytical record for lots 1R through 5R refer to Reference #3701.

Examination of the laboratory notebooks referenced on the analytical records revealed other possible discrepancies. For example, the analytical record A-9129 for DKP Lot 5R showed an assay (titration) of 100.0 percent. The analytical record referenced two different lab notebooks assigned to two different analysts. Examination

of lab notebook AR-68 assigned to Sandra Ann Carey revealed that she had analyzed 3 samples of lot 5R on 11/9/72. Results of the analysis showed that sample one had an assay (by titration) of 89.70 percent, sample two, 87.93 percent and sample three was discarded.

Apparently not satisfied with her results she repeated the assay on the same day (11/9/72) and obtain 93.23 percent (the average of 3 samples), still well below the specification of 99.0 percent. The other lab notebook referenced was AR-57, assigned to E. Aranda. This notebook showed that analyst Aranda performed an assay (titration) of lot 5R on 12/1/72 the results of which were 114.83 percent for 3 samples. Apparently not satisfied with the results, he repeated the assay on 12/6/72 and obtained 100.4, 99.9 and 99.8 percent for an average of 100.0 percent. This result (100.0 percent) was the only one reported on the analytical record A-9129.

The analytical record (A-7291) for DKP lot R shows a result of "less than 20 PPM" for the heavy metals test. Two laboratory notebooks are referenced: VSH-I, pages 260-263, and AR-23, page 269. Examination of both of these books revealed no mention of a heavy metals test.

The analytical record (A-9805) for DKP lot 6R (JDR-5-30A) also showed a result of "less than 20 PPM" for heavy metals test. Examination of the referenced laboratory notebook (AR-77, page 83-86) revealed no evidence of a test for heavy metals.

The analytical record (A-9829) for DKP lot 7R (JDR-5-30B) again showed "less than 20 PPM" heavy metals. Examination of the referenced lab notebook (AR-93) again showed no evidence of a heavy metals test.

The above discrepancies were the only ones noted with respect to lots 1R through 7R of DKP. All other criteria for identity and purity of DKP as shown in the reports of analysis, conforms to Searle specification sheet marked "tentative specifications for SC-19192". It should be noted however that none of the seven lots of DKP met the specifications on the sheet dated 12/4/69, with respect to melting range.

STABILITY AND HOMOGENEITY OF DIET MIXTURES

A stability study was initiated in January 1972, 2 months after the rat study (E-77/78) had begun. The objective of the study

was to evaluate the stability of SC-19192 (DKP) when mixed with Rockland mouse/rat diet and held at room temperature (73 degrees F.). Two concentrations of diet mixture were tested: 3.0% and 6.0% DKP. A preliminary analysis was performed on 1-31-72 to test the analytical method (T.L.C.), and recovery of DKP. Assays were performed at one-week intervals on 2-16-72, 2-23-72, 3-1-72, 3-8-72, 3-15-72, 3-23-72, and 3-29-72. Copies of all analytical reports were obtained and are attached to this report, along with a copy of the protocol. (see exhibits #24-27).

The titration method of DKP analysis was used initially, along with the TLC method. The titration method was discontinued after the 1-week analysis on 2.-23-72. Thin layer chromatography was used thereafter. It should be noted that the titration method was the only reliable quantification method for DKP analysis.

Page #54 of the laboratory notebook #51 (See Exhibit #26) indicated (from the photograph) that there was something in the basal diet itself producing a spot on the TLC plate which had an Rf. value corresponding to DKP. This would make quantification of DKP by this method difficult.

Some of the photographs of the TLC plates approached to laboratory notebook #51 showed no DKP reference standards. The analysis described on pages #69-72 did use a DKP standard but those on pages #88-89, #106-107, #144-145, and #284-285 showed no reference standard. (See Exhibit #26)

Only one solvent system was used for development of TLC plates throughout the study, even though it was apparent that some material in the basal diet was producing a spot on the TLC plate with an Rf. value corresponding to DKP. with the above method of analysis, only materials reacting with the potassium iodine starch reagent would be detected. Another solvent system was available for TLC analysis of DKP (See Exhibit #19) but apparently was not used in the stability study.

It should also be noted that some of the chromatograms showed poor separation (day 28 on pages #144-145, and day 35 on pages #156-157 of notebook #51). See Exhibit #26)

In general, the data described in the reports of analysis corresponded well with the laboratory notebooks, although the poor chromatograms were not mentioned in the reports of analysis.

The level of impurities as indicated by TLC was low; the major impurity, an unknown substance, represented about 2% of the DKP. The remaining impurities were also low, as apparent from the density of the TLC spots compared with the DKP spots, but were not quantified.

A glossary of terms for aspartame and its diketopiperazine is attached as exhibit #9 and copies of specifications for DKP are attached as exhibits #16-18.

No homogeneity tests were performed on any batches of diet mix used in E-77-78, and evidence exists that homogeneity was a problem with the DKP diet mixtures. Two of the stability study assay reports, analytical numbers A7728 and A7739 both dated 2-16-72, contained the statement: "These samples were not homogeneous. They had to be reground before they could be sampled". The assay reports were signed by Barbara Bickford, a Searle analyst.

We examined the laboratory notebook #51 assigned to Barbara Bickford and noted that a B & W polaroid photograph of the non-homogeneous sample in question was attached to page #58 of the notebook. The photograph clearly shows discrete lighter colored particles of diverse size and shape distributed nonuniformly throughout the mixture. These lighter colored particles appear to be distinct from the fairly fine granular nature of the chow itself.

A copy of this photograph was made and is attached to the report as exhibit #29. When questioned about the size of the white square sheet of paper in the photograph (on which the diet mixture was placed) Ms. Bickford and C. Seul both stated that it was 6"by6", when we interviewed them on 6-2-77. When the photograph was enlarged until the sample paper was 6"by6" (actual size) we measured the large particles (which were identified as DKP by Ms. Bickford) and found them to be 4 to 6mm in size.

When we interviewed Ms. Bickford on 6-1 and 6-2-77, she stated that she had nothing to do with the preparation of the diet mixtures. She said that the samples had probably been received from the toxicology lab and stored at room temperature. Her procedure was to weigh out a predetermined amount of the sample, and if not a uniform powder she would re-grind it with a mortar and pestle, and would make a note of this in her lab notebook. We asked Ms. Bickford if she ever reported this lack of homogeneity to Dr. Rao, and she replied that she did not.

We could not determine whether the samples assayed in the stability study were from diet mixtures actually fed to the animals, in spite of the fact that we were told so by some employees.

On 6-2-77, we interviewed Analyst Barbara Bickford and Clifford Seul, who was Mrs. Bickford's supervisor at the time that the stability samples were analyzed (Feb. 16, 1972). Clifford Seul told us that the samples analyzed on 2-16-72 and described on page #58 of laboratory notebook #51, were obtained from the admixture being fed the rats on study, and not a special mixture prepared for the stability study.

On 6-1-77 we interviewed Bart Tangonan, whose duties included observing, weighing, and feeding the animals, and mixing the diet for study E-77/78. Mr. Tangonan did not remember if there were any written instructions for mixing the diets but thought that it was mixed for a specified length of time. He said that if the diet appeared to need more mixing, it was mixed longer. He could not remember anything about the samples obtained for the stability study.

On 6-3-77 we interviewed Tony Martinez who was a supervisor in the Toxicology Laboratory in 1972. He told us that although the analytical report indicated that the sample was submitted by Dr. Rao, actually anyone in the toxicology laboratory could have submitted the sample. According to Mr. Martinez, the normal procedure in such cases was to collect a sample just after mixing compound and diet and then repeat this in four weeks. He could not specifically recall what was done with regard to the stability study in question, and could not remember whether the samples had been taken from the diets being fed the animals on study P.T. 98873 (E-77/78). He did not remember any problems with mixing, but did say that a longer mixing time was required at higher compound concentrations.

A point to be considered, however, is that although the analytical report states that the material analyzed was prepared to contain 3.0 and 6.0% DKP, none of the diets reported to be fed contained these exact amounts of DKP according to the records of food concentration calculations, which were used to prepare the diets for study #E-77/78. (see chart attached to Exhibit #30.) In addition, the stability study protocol (Exhibit #24) specified that the test batches would be 1 kg. in size. If the protocol was followed, the small (1 kg.) test batches would not have been sufficient in size to feed a single dose group of the animals on study. (See Protocol, Exhibit #24).

Additional evidence of homogeneity problems was revealed when a former Searle employee, Raymond Schroeder, was interviewed by the other FDA team on 6-22-77 concerning teratology studies E-5 and E-89. At that time Mr. Schroeder volunteered the information that homogeneity may have been a problem in the DKP diet mixtures, but not in the aspartame diet mixtures. A follow-up phone call to Mr. Schroeder was made on 7-13-77, and at that time he stated that he observed the DKP diet mixtures being fed to the animals, and that in his opinion, the particles of DKP were large enough to allow the rats to discriminate between the DKP and basal diet. (See Thomas F. X. Collins memos (2) dated 7-14-77 (attached as Exhibit #31). An interview was arranged for July 18, 1977 between Mr. Schroeder and members of the FDA team investigating study E-77/78. The interview was conducted at [information blanked out to protect the individual], Mr. Schroeder's current place of employment. Also participating in the interview by means of a conference phone were Thomas F.X. Collins, and Leonard Friedman. Mr. Schroeder stated that he was not certain of the date, or even the year, when he observed the rats being fed DKP diets. He further stated that he could not be absolutely certain that the rats he observed were on study E-77/78. He was not certain about the dose levels of the diets he observed, and could not remember how many times he observed the DKP diets. He estimated that he observed the DKP diets "one or two times". When he was shown an actual-size enlargement of the DKP diet mixture (See Exhibit #29) he stated that to the best of his knowledge, the white particles that he observed were not as large as the largest particles in the photo, but may have been similar to the smaller white particles. He said that he may have mentioned the appearance of the DKP diets to Dr. Rao.

Mr. Schroeder seemed reluctant to make any positive statements during this interview. Dr. Collins reminded Mr. Schroeder that he had previously volunteered the information that the DKP diets appeared to be non-homogeneous and that the rats could probably discriminate between the DKP particles and the basal diet. Mr. Schroeder replied that he had had some time to think over his previous statements and now wasn't sure about them. He told us that there must be people at Searle who know more about the DKP diets than he did. (see memo dated 7-19-77, attached as exhibit #32, which describes our interview with Mr. Schroeder).

When we arrived at [address expunged] on 7-18-77 at approximately 2:40 P.M., we were asked by the receptionist to sign a log book. While signing the log, we noted that a G.D. Searle employee (W. R. Pool) had signed in on 7-15-77. W. R. Pool works in the Toxicology Section (Safety Assessment Division) at Searle Laboratories.

During our interview, we asked Mr. Schroeder if he had been contacted by anyone from Searle during the period from June 22, 1977-July 18, 1977. He replied that he had not.

We again interviewed Tony Martinez on 7-19-77, and specifically asked him if he was aware of any homogeneity problems with the DKP diet mixtures fed the rats in study #988S73 (E-77/78). He replied that he was not aware of any problems. We asked whether any samples of DKP had been retained by Searle Laboratories. We were told that a small quantity of DKP remained in the compound file, but that it was a lot other than those used in study E-77/78. Upon request, we were then shown a jar containing 4.9 grams of DKP, lot #TJT-12-32. Its appearance was that of a fine white crystalline material with a tendency to adhere to the sides of the jar. Mr. Martinez said that this was the only lot of DKP remaining at Searle.

We also interviewed Teratologist Alan Mitchell, on 7-19-77. We had previously noticed his name on one of the DKP compound inventory cards, and his name had also been mentioned by Raymond Schroeder, in connection with DKP. Mr. Mitchell stated that he had done two teratology studies with DKP, both with rats, and both in 1972. In one study the DKP was administered I.G. (as a suspension), and the other was a dietary feeding study. Mr. Mitchell told us that he didn't recall any problems with homogeneity in the dietary feeding study. He said he never remixed or reground any DKP diets. He admitted, however, that when he prepared the diet mixtures, he first sifted the DKP through a hand flour sifter.

We attempted to interview a former Searle employee, Dr. Rao, after learning that he still lived in the Chicago area. Dr. Rao had been in charge of the DKP stability study and was the monitor for study E-77/78. After reaching Dr. Rao by telephone on July 25, 1977 he stated that he would like to talk to his attorney before consenting to the interview. We then received a call from his attorney, Mr. John H. Bickley, Jr., who told us that the interview would be of no advantage to his client, and he therefore refused to allow it. A memo

of telephone conversation between J. Bressler and Mr. Bickley is attached as Exhibit #33.

CALCULATING DIET CONCENTRATION & BLENDING OF TREATMENT MIXTURES

There were no batch records to show the quantities of DKP and basal diet weighed, type of mixer used, mixing time, dates, or names of individuals performing the weighing and blending operations. We were told that mixing was performed in a Hobart mixer, and that mixing times were about 10 minutes. There was no evidence that any tests had been done to determine the blending characteristics of the mixer, or to validate the 10 minute mix time. Fresh batches were mixed on a weekly, bi-weekly, or monthly basis, and batch size ranged from 6 kilograms to 28 kilograms during the study.

The concentration of DKP in the basal diet was calculated by the Mat-Stat Department on a weekly, bi-weekly, or monthly basis (based on the food consumption for the previous time period), and submitted to the Path-Tox Department as a Food Concentration Prediction record. The concentration was expressed as grams of DKP per kilogram of basal diet. The Path-Tox Department Personnel then multiplied the grams of compound indicated on the prediction record by the number of kilograms of diet mix needed to arrive at the proper quantities of DKP and basal diet to be blended. The concentrations were calculated to yield the proper dosage levels of 9.75, 1.5, and 3.0 grams of DKP per kilograms of body weight per day, for the low, medium, and high dose groups. (Copies of Diet Calculation Records are attached as Exhibit #34). At the end of each treatment period, the remaining treatment mixtures were discarded and fresh batches were made.

Now reserve sample of either the DKP or the DKP/diet mixtures used in this study were retained according to Searle.

DKP was withdrawn from stock by means of a compound inventory card, which was filled out by the person requesting the material. Tony Martinez was the person that usually requested DKP for use in study E-77/78. We examined eighteen (18) compound inventory cards which accounted for 177.0 kg of DKP withdrawn from stock. According to our calculations a total of 152.81 kg of DKP would have been necessary

achieve the diet concentrations and batch sizes that were reportedly used in the study. A total of 230.0 kg of DKP was manufactured by Searle Chemist Jack Drogt. Following are tables showing the quantities of DKP manufactured, calculated quantity required for the study, and quantities withdrawn from stock.

QUANTITIES MANUFACTURED

Lot #.	Quantity (After Milling)
1R	
2R	
3R	
4R	Another FDA gutted table!
5R	
6R	
7R	

Total

CALCULATED QUANTITIES REQUIRED FOR THE STUDY

Dose Group	Calculated Quantity Required
Low Dose Males	
Mid Dose Males	
High Dose Males	
Low Dose Females	Another FDA gutted table!
Mid Dose Females	
High Dose Females	

QUANTITIES WITHDRAWN FROM STOCK (FROM COMPOUND INVENTORY CARDS)

Date Withdrawn From Stock	Quantity	Lot
10/29/71	kg	1R
1/4/72	kg	1R
2/28/72	kg	4R
3/11/72	kg	3R
3/29/72	kg	2R
9/11/72	kg	3R
10/10/72	kg	2R
*	kg	2R
12/1/72	kg	3R
*	kg	4R
12/27/72	kg	5R
*	kg	2R
1/25/73	kg	5R
3/22/73	kg	6R
4/18/73	kg	5R
7/10/73)5 kg	6R
8/10/73)5 kg	6R
9/7/73	kg	6R
11/2/73	kg	7R
Another FDA gutted table!		
TOTAL		kg

* These three cards were not signed or dated.

It should be noted that only two of the 18 compound inventory cards specified that the SKP withdrawn from stock was to be used in study E-77/78 (PT 988S73). Thirteen of the cards list "Toxicity" or "Toxicology" as the reason for withdrawal. Three of the cards have no entries at all, except for the word "empty". (Copies of the compound inventory cards are attached as Exhibit #28).

The total quantity withdrawn from stock is kg in excess of the amount necessary to achieve the diet concentrations used in the study. (Based on the diet calculation records attached as Exhibit #34, which we used to construct the diet calculation summary table attached as Exhibit #30).

It is not known whether any of the kg of DKP accounted for on the 18 compound inventory cards was withdrawn for use in studies other than E-77/78. We could find no other records to verify the amount of DKP withdrawn for, or used in this study.

ANIMALS UNDER TEST

Three hundred and sixty weanling albino rats, CD strain, 180 of each sex, were used. The rats were 21 days old when received from the
Copies of shipping labels were obtained, and are attached as Exhibit #10.

The rats were housed individually in wire cages and were given a one-week acclimation period before being placed on treatment at the age of four weeks.

Rockland Rat/Mouse Diet (complete), , was fed for the first 62 weeks, and /Rat Chow was used from week 63 until the study was terminated at 114 weeks.

The animals were housed in air conditioned rooms maintained at 72 degrees F, with artificial fluorescent lighting at 12 hours per day exposure.

The rats were divided into 12 housing groups, (6 groups per sex), 30 rats in each housing group. Initiation of treatment was staggered over a 2 week period, beginning 11/8/71.

Each housing group was composed of dosage groups as follows:

Treatment Group	Dosage gm/kg/day	Multiples of Estimated Daily Human Dosage	No. of Rats Per Housing Group		Total Rats
			Male	Female	
Control					
Low					
Medium					
High					

This entire table was blotted out prior to delivery

RANDOMIZATION OF ANIMALS

Computer-generated randomization tables were used for assigning the dose and housing groups (copies of these tables were obtained and are attached as Exhibit #6). Each housing group consisted of 30 animals (12 controls, 6 low, 6 medium, and 6 high dose). Each animal was assigned a letter to

designate the housing group (A through M), a cage number (1 through 30), a letter to indicate dose group (C,L,M & H), and a letter to indicate sex (M or F). For example, animal A30CM would be a control male, in housing group A, occupying cage number 30 (Exhibit #69).

Each rack (30 animals) contained a random distribution of control and treated animals. An example of a typical housing group is shown in the diagram attached as Exhibit #7.

The specific problems of feeding animals housed in the above manner were discussed in the report generated by the task force investigation of Aspartame in 1975/1976. We will reiterate them here:

Housing experimental animals in this manner (controls, low, medium, & high dose animals randomly distributed on the same rack) would greatly increase the chance of administering the wrong diet to the animals. The chance of error was compounded by the method used to feed the animals which was as follows: At the specified intervals, the animals were weighed, and the empty food jars were removed, weighed and new food jars placed in the cages. The new (filled) food jars were placed on a mobile cart in rows corresponding to dose group (See Exhibit #8). The cart was wheeled to the Intec Unit and placed up against it with the rows of high dose jars farthest away from the operator. The operator started from the upper left corner of the housing rack, (See Exhibit #7), removed the mylar card from the cage and inserted it into the Intec Unit. This printed out the animal's identification number. A color coded card for dose level, also bearing the animal number, remained on the cage. The technician then opened the cage, removed the animal, placed it on the scale pan, pushed the button to register the weight and returned the animal to its cage. He then removed the empty food jar, placed it on the scale and pushed the button to record the empty feeder weight. The empty jar was placed on another mobile cart provided for that purpose. The new (filled) jar was selected from the appropriate row according to dose level (Exhibit #8), placed on the scale, weight recorded, and the jar placed in the cage. The Card was then removed from the unit and replaced on the cage.

This procedure was repeated for all cages proceeding from left to right. It is important to note that none of the food jars were identified in any manner, as to animal number or dose level. The position of the jar on the cart was the only means of identifying the proper dose level.

This procedure was used when concentrations were changed. At other times, the feeder jars were weighed; filled with the appropriate diet/dose, weighed and replaced in the cage. If a feed container was almost empty, or contained feces, it would be replaced with a new container.

IDENTIFICATION OF ANIMALS

No ear clips or other methods of uniquely identifying each animal were used. Animals were individually housed (one animal per cage) and a color coded card containing the cage number, compound number, project number (Path-Tox No.), and dose level was attached to each cage. When an animal died during the study, the color coded identification card was removed from the cage and accompanied the animal to the necropsy laboratory.

Also attached to each cage was a Mylar Card which identified the animals for the Computer System.

At the time of death or sacrifice the animals were assigned a pathology number which was used to identify the animal during tissue processing, and on all records pertaining to pathology. The pathology number was a five-digit sequential number assigned by the pathology department. An example of a pathology number is 94.893. The number was etched on all slides as a permanent identification.

All animals that died during the study were assigned an additional number called a "Master Number". The master numbers denote the chronological order in which the animals died during the study, by dose group. For example, CM38 would be the 38th Control Male to die during the study. Master numbers were noted only on the gross pathology sheets and not recorded for all animals. Four animals that died during the study had no master numbers. We were not able to locate any other records providing the missing numbers. Also, two of the master numbers (CM28 and CM29) were out of sequence.

A chart was made by the FDA team, which shows the animal number (cage #), pathology number, master number, and the complete pathology history of each animal. The chart is organized by dose group, and is attached as Exhibit #35.

ANTE-MORTEM OBSERVATIONS

"Observations for Drug Effects" records are attached as exhibits #70 & 71. These records were completed on a weekly basis for the first four weeks, every two weeks through week 12, and every 4 weeks thereafter. Each record lists the animals in a specific housing group, and entries are made for the following parameters: appearance and awareness, rales, eyes, motor activity sensory loss, urine/feces, appetite/thirst, and tissue masses/lesions. There is also a space for notes, and masses are routinely described on the reverse side of the sheet. The top of the sheet has blocks for entering the date of observations, number of weeks on treatment, and signature or initials of the person making the observations. It was noted that many of the observation records were not signed or initiated. Following is a tabulation of the numbers of records in which the person making the observations is not identified.

HOUSING GROUP

NO. OF RECORDS NOT SIGNED OR INITIALED

A	6	(wks 80, 92, 84, 88, 93, 94)
B	6	(wks 80, 84, 88, 92, 93, 96)
C	5	(wks 80, 84, 88, 92, 96)
D	7	(wks 79, 80, 84, 88, 92, 94, 96)
E	6	(wks 76, 80, 84, 88, 92, 96)
F	9	(wks 76, 77, 80, 81, 84, 88, 92, 93, 96)
G	5	(wks 80, 84, 88, 92, 96)
H	11	(wks 1, 68, 76, 80, 81, 83, 84, 88, 92, 95, 96)
J	6	(wks 76, 80, 84, 88, 92, 96)
K	8	(wks 71, 76, 80, 84, 88, 92, 93, 96)
L	5	(wks 76, 80, 84, 92, 96)
M	5	(wks 76, 80, 84, 92, 96)
TOTAL	79	records not signed or initialed

In addition to the lack of signatures, it was noted that many of the records were not originals, but appeared to be **xerox copies of the originals**. Surprisingly, some of the xerox copies had "original"

initials. It was obvious that the initials had been placed on these sheets sometime after the sheets had been filled out, and after they had been copied.

Some examples of discrepancies of this type are as follows:

- 1.) In housing group A, 26 of the 39 observation records were xerox copies with original initials.
- 2.) In housing group B, 27 of the 43 observation records were xerox copies with original initials.
- 3.) The record for week 76 of housing group A was a xerox copy but the date, initials, an week are all original.
- 4.) For week 96 of housing group K, both an original and xerox copy of the observation record are present. The xerox copy has original initials and a "B" entered in the "tissue masses and lesions" column. There is also an entry in the "notes" column for rat #K25CF. The original record also has a "B" entered in the "notes" column. All of the above entries had obviously been made sometime after their original record had been completed and the xerox copy made.
- 5.) A record dated 4-27-73 for housing group M does not have the date entered. The observations were made for this animal on sheets covering weeks 92, 96, 100 and 104, indicating that the animal was dead. The record for week 108, however, shows that the animal is alive, with motor activity, appetite, and thirst. The record for week 112 again shows that the animal is dead.

In addition to the discrepancy noted above, there is also an obvious error in the dating of these records; the observation sheet for week 92 is dated June 13, 1973, and the observation sheet for week 88 is dated July 16, 1973.

Ante-mortem observations were also made on other types of records. A volume entitled "Tissue Masses and Deaths" (exhibit #65) has a record of the date that each animal died during the study. The deaths are recorded in two different ways in this volume. One record has a chronological list of deaths, and another record has a list of deaths organized by housing

group. This volume also has a "palpation Record", which describes each tissue mass, and lists the date that it was initially detected.

It was noted that many of the animals in the sequential record of deaths were listed out of sequence. Following is a tabulation of the animals that were out of sequence.

(* indicates animal is listed out of sequence)

ANIMAL NO.	DATE FOUND DEAD	NO. OF DAYS ON TREATMENT
E6HN	8-12-73	640
E24HM	8-16-73	640
G10LM	*8-14-73	638
L11CM	5-6-73	535
C17CM	*5-4-73	542
A14MM	5-21-73	560
A24HM	10-15-73	707
C14HM	*10-27-73	718
E22CM	10-19-73	707
E29LM	10-25-73	714
A4CM	10-26-73	718
E26CM	11-15-73	735
C12LM	*11-14-73	736
L20CM	11-19-73	732
A11CM	11-25-73	748
A18CM	*11-18-73	741
J19MM	12-10-73	754
F25HF	6-10-73	576
D21CF	*6-9-73	577
H6CF	6-17-73	579
M12CF	*6-16-73	575
F18CF	7-22-73	615
H22LF	*8-20-73	641
D25MF	7-25-73	623
G2LM	10-5-73	689
G7CM	*6-14-73	567

The log of animal deaths in the "tissue masses and deaths" book was considered the primary data. Animals were allegedly recorded in this book as they died. When the above discrepancies were pointed out to Searle personnel, we were told by Tony Martinez on 4-28-77 that this log was compiled from the body and feeder weight data. When it was pointed out that the exact date of death could not be determined from the body and feeder weight data, or from the observation records, we were told that the primary record of animal deaths was the log organized by Housing Group in the "Tissue Masses and Deaths" book. We were told that the chronological log of animal deaths was made by transferring the data from the log organized by housing group, and therefore the animals being recorded out of sequence was not significant.

We then pointed out that the chronological record of deaths also contained the date fixed "in toto" and the date autopsied, neither of which were found on the log organized by housing group. If the data was transferred from one record to the other, we wondered where the "date fixed in toto" and "date autopsied" came from. We posed this question to Searle personnel and we were finally told on 4-29-77 that the "fixed in toto" and "date autopsied" columns on the chronological record of deaths was considered to be primary data, although it was prepared simultaneously with the pathology sheets, which contained the same data.

Another source of ante-mortem data was the ophthalmoscopic examination record. Dr. Youkilis performed the eye examinations at periodic intervals and recorded his findings on the ophthalmoscopic record sheets. These eye exam sheets were eventually attached to the pathology records, and the results of the eye exam was incorporated into the Clinical History of the animal on the pathology sheet.

During our review of the pathology records we noted that there were no eye exam sheets for 15 animals, all of which had been included in the individual Ophthalmoscopic Findings in the submission to FDA (Vol. 1, table 1, pages 122-133). All of these missing eye exam sheets were for animals that had died during the study. On 6-30-77 we interviewed Donna Helms, who told us that she would make an attempt to find the missing records. We advised her that Dr. R. Stejskal had told us on 6-29-77 that not all of the eye exam sheets were attached to the pathology records, and that there may be another file of eye exam sheets somewhere.

On 7-1-77 Donna Helms reported that she had found the missing eye exam sheets in the K-1 File Room in K-Building. After reviewing these records we found that a few discrepancies still existed. They are as follows:

- 1.) It appears that animal J3CM on page 125, Vol. 1 of the submission to FDA is in error. We could find no records to substantiate the **listed corneal scar and haziness for this animals**. Also, the observation records indicate that J3CM died at week 78. It appears that the correct animal on page 125 should be J2CM and not J3CM.
- 2.) We found eye exam sheets for H26MF and J29CM, yet the findings were not reported in the submission to FDA.
- 3.) There seems to be a discrepancy between G16CM and G12CM. The pathology sheets for both of these animals report the identical ophthalmoscopic finding, yet there is no eye exam sheet for G12CM, and only the finding for G16CM was reported in the submission to FDA (Vol. 1, p. 125).

During our data review, we found an internal memo from Dr. Youkilis to K.S. Rao, dated 4-28-74. The text of this memo is as follows: "

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Note... **This entire memo was expunged from the delivered document, by parties unknown.**

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A copy of the above memo is included in exhibit #72. Dr. Rao and Dr. Youkilis are no longer employed by Searle.

When an animal spilled an excessive amount of food, this was noted on the observation records by means of an asterisk in the "appetite/thirst" column. The asterisk was also used to denote food spillage on the Teletype sheets for body/feeder weight data. The amount of food spillage was not quantitatively determined by the technicians assigned to observe, feed and weigh the animals, but we were told that they made an effort to return spilled food to the food cups whenever possible. WE were also told that food consumption data for those rats marked with an asterisk on the body/feeder weight sheets was not used in Searle's statistical analysis of the data.

The "palpation record" in the "Tissue Masses and Deaths" volume shows that **tissue masses were sometimes excised from the animals**. The record indicates that a tissue mass measuring 1.5x1.0cm was excised from animal B31HF on 2-10-72. The record also shows that a "skin incision over mass" was performed on animals C22LM and G25LM on Feb. 10, 1972.

DOSAGE, BODY WEIGHT AND FOOD CONSUMPTION

DKP levels for the feeding study were multiples of 100, 200 and 400 times the estimated human dose. The levels in g DKP/kg body weight/day were 0,0.75, 1.5 and 3.0 for the control, low, medium and high treatment groups, respectively. The doses were mixed in the diet as described in Calculating Diet Concentration and Blending of Treatment Mixtures.

Individual body weights were recorded weekly for the first four weeks, once every two weeks for the next eight weeks and once every four weeks thereafter. The amount of food consumed was measured every week. An automated weighing system was employed consisting of an Intec balance and a Teletype machine. The Teletype produces a typewritten sheet and a machine-readable punched paper tape. All the typewritten sheets for the study were available. Xerox copies of these sheets were taken to the Division of Mathematics and technical Operations Staff of the form and calculated by a computer program designed by Dennis Wilson, Division of Mathematics.

In designing the computer program it was necessary to make certain assumptions on the handling of the data. One assumption concerned missing data, e.g. the empty feed cups weights were missing for the "D" housing group at the 12th week. Dr. George Clay, Group Leader, CNS Pharmacology, Searle and scientific co-ordinator for the FDA team, was unable to determine whether these animals were omitted from the food consumption calculations for that week, or whether the data for these animals from

the 11th and 13th weeks were averaged and the average substituted for the missing data. Employees of Searle's Math-Stat Department who had worked on the program for this experiment are no longer with the company. Dr. Clay calculated a few of the figures from the 11th and 13th weeks and stated that it appeared that the data had been averaged. For the FDA recalculation it was chosen to omit the animals with the missing weights from the calculations. In several instances (For example, C group males, mid and high levels for the 13th week; A group males, high level for the 99th week) the dietary concentration shown on the weight sheets did not agree with the concentration listed for that level in the other housing groups. Dr. Clay assured us that all the animals of the same sex in a given experimental group received the same dose for the same week on the experiment. He also assured us that the Searle computer program did not pick up the doses from the weight sheets. In the FDA program, the dietary concentrations were taken from the diet calculation sheets (Exhibit #34). Certain animals on the raw data sheets were marked with an asterisk. Dr. Clay explained that the asterisk indicated spillage and such animals were omitted from the food consumption calculations. This practice was followed in the FD computer program. In calculating the food consumption (g food eaten/day/ kg body weight) and the dosage (mg test compound/day/kg body weight), the body weight used was the weight at the end of the period under consideration, i.e. the current weight.

In addition to the calculations which were included in the Searle submission, the FDA program included calculation of the actual amount of food ingested, i.e., the total amount of diet ingested minus the test compound, and of the food efficiency (g weight gained/100 g actual food eaten). The food efficiency was calculated in order to determine whether the volume of DKP in the diet (which exceeded 7% of the diet for the high dose males at intervals during the study) was contributing to the body weight depression seen with DKP. This explanation of the body weight depression was discussed by Dr. John H. Rust, a Searle consultant, in a memo dated April 5, 1976 to Dr. R. McConnell; in a memo to the file dated September 30, 1974 by Dr. McConnell; and in a memo to Dr. K. S. Rao dated August 29, 1974 by Dr. G. L. Schoenhard. (Exhibits #36-38).

The average body weights and weight gain (% change/week) from the FDA analysis of the Searle raw data are presented in Table 1 (Exhibit #39) which corresponds to Table 3 of the Searle submission.

Weights which differ from the Searle submission by one (1) g or more and weight gains by 0.1 percentage point, or more are underlined. Fifteen differences were noted as follows:

Average Body Weight Discrepancies

Days	Sex	Dose Level	Searle Submission	Calculated
280	M	M	591.7	589.2
364	F	L	353.2	345.1
420	M	M	613.2	614.4
700	M	C	595.4	579.3
728	M	C	594.4	597.2
728	F	H	343.1	341.2
784	F	C	453.4	459.9

Percent Weight Gain Discrepancies

Days	Sex	Dose Level	Searle Submission	Calculated
14	M	C	35.11	35.23
21	M	C	22.80	22.69
280	M	M	0.33	0.21
364	F	L	-0.16	0.04
392	F	L	1.13	0.85
728	F	H	0.32	-0.18
756	F	H	-0.14	0.08
805	F	C	-0.04	-0.39

The food intake (in g/day and in g/kg/day) and dosage (in mg/kg/day) from the FDA analysis are presented in Table 2. This table corresponds to Table 4 of the Searle submission. There are numerous discrepancies (in excess of 80) of one (1) gram or greater in the food intake expressed in grams/day. Many of the discrepancies are probably the result of an error in the Searle computer program (see Exhibit #76). Through this error there was a failure to adjust the food intake for the precise number of days between weighings for the individual housing groups. This programming error had been pointed out to Searle by the Task Force but no amendment to the Searle submission was made. There are more than forty discrepancies of 5 or more grams when the food intake is expressed in g/kg/day. The Searle programming error would contribute to discrepancies in this expression of the food intake. The use of the current body weight in the FD analysis may also be a contributing factor. Most of the dosage

calculations from the FDA program differ from the Searle submission by 10 or more mg. The two factors of the Searle programming error and the use of the current body weight in the FDA analysis would contribute to discrepancies between the FDA analysis and the Searle submission. Despite the discrepancies the FDA analysis shows dosage levels corresponding to the intended levels of 0.75, 1.5 and 3.0 g/kg/day. The test compound would have to be homogeneously mixed into the basal diet in order for these calculated dosage levels to be actually consumed. All discrepancies between the Searle submission and the FDA analysis shown in Tables 1 and 2 are underlined.

Table 3 presents the food efficiency (g gained/100 g actual food consumed) calculated in the FDA analysis. There is no corresponding table in the Searle submission. Tables 1, 2 and 3 and the computer printout of the FDA analysis are Exhibits # 39-42. Statistical analysis of the body weight and food consumption data was made and is shown as exhibit #73.

ORGAN WEIGHTS

Organ Weights were entered on the gross pathology sheets at the time of autopsy. We compared all of the individual organ weights on appendix table 5 in the submission to FDA (Vol 1, pp. 222-226) with the original data on the gross pathology sheets. A total of eleven (11) errors were noted in transcribing the raw data from the pathology sheets, to the tables in the submission to FDA.

The errors are tabulated below:

Animal No.	Organ	Wt. Shown In Submission	Wt. Recorded on Original Pathology Sheet
A12CM	Kidneys	3.75 G	3.45 G
L28LM	Ven. Prostrate	747 mg.	474.7 mg.
C01MM	Kidneys	9.40 G	9.219 G.
C02HM	Kidneys	1.46 G	4.259 G
E14HM	Kidneys	11.74 G	4.746 G
J12HM	Pituitary	3.0 mg.	3.3 mg.
J30HM	Ven. Prostrate	444 mg.	444.8 mg.
F17CF	Ovaries	36.7 mg.	233.5 & 36.7 mg.
H30CF	Liver	9.4 G	9.493 G
B20HF	Uterus	1115 mg.	1155 mg.
K11HF	Adrenals	799.1 mg.	797.1 mg.

Copies of the applicable pages of the submission, appendix table 5, with errors indicated, are attached along with copies of the gross pathology sheets documenting the errors. (See exhibit #83)

DISEASES

The submission to FDA (Vol. 1, P. 10) reported that an unidentified infectious disease spread among the animals between 12 and 14 weeks of treatment, and that a second unidentified infectious disease occurred in high incidence between 48 and 52 weeks of treatment. In both cases, the control and treated rats were reportedly affected with equal frequency and severity. The same page of the submission also stated that over a period of two weeks, a total of 17 animals (8 control, 3 low dose, 4 medium dose, and 2 high dose) died. A memorandum dated October 13, 1972, and that more animals were morbid. Dr. Rao reported that this primary antemortem symptom observed was inappetance and labored respiration. Postmortem examination of dead animals revealed primary lesions in the lungs, and lungs exhibited patchy pneumonia, according to Dr. Rao. The memo indicates that Dr. Rao intended to administer 10,000 units of penicillin G, intramuscularly, to all the animals 2 to 3 times per day beginning 10/30/72. A copy of Dr. Rao's memo is attached to the protocol (See Exhibit #77, Section 1).

The submission to FDA (Vol. 1, P. 10) stated that, "to prevent further loss of animals, all morbid rats were injected IN with 20,000 units of potassium penicillin G daily for 4-8 days."

A review of the injection records (attached to Vol. A of Exhibit #75) showed that some animals were treated between approximately 51 and 60 weeks, and in one instance, a high dose animal, kB3HF, received at least 10 injections. In addition, some animals received 30,000 units per day (10,000 units 3 times per day) rather than the 20,000 units reported in the submission.

The records also indicated that penicillin was administered to four rats beginning on May 16, 1973, and continued daily through May 28, 1973. This third occurrence of infectious disease and penicillin administration was not reported in the submission to FDA.

SURVIVAL

An attempt was made to construct a Survival Table using data from the "Tissue Masses and Deaths" book.

We were unable to determine the exact method used in constructing the table in the FDA submission. There was some survival data in the "Tissue Masses and Deaths" book (Exhibit 65), but this only extended through week 109 and consisted solely of running totals. According to Tony Martinez deaths purportedly were initially recorded in any one of the following documents:

- (1) Body/Feeder Weight Sheets
- (2) Autopsy/Pathology Sheets
- (3) Observation Sheets
- (4) Palpable Mass Sheet

He said that animals found dead at feeding/observation intervals were usually recorded on the observation, or Body/Feeder Weight Sheets. At other times, the death was recorded on a "scrap" of paper and then later transcribed to one of the documents. The term "scrap of paper" was used by Searle personnel both during the Task Force and current investigations. No notebooks containing observations or deaths ever surfaced during either investigation. Animals killed "in extremis" were recorded on Autopsy sheets. The least likely source for original death recording would be the Body/Feeder Weight Sheets.

Dates of death sometimes differed on the various records, making it impossible to determine which one was correct. A survival table was finally constructed for weeks 40-115, using the Body/Feeder weight teletype (hard copy) sheets and dates on which animals no longer appeared as a base (Exhibit 68). In this manner, the number of days on study was calculated for each animal (Exhibit 66). Using starting dates for each group, a calendar was made to encompass the entire duration of this study (Exhibit 67). Toward the end of the study, some feedings/observations were made at intervals such as 109 3/7, 110 6/7 and 111 6/7 weeks, so some differences are anticipated between this table and the one in the FDA submission. However, the final number of animals in each dosage group and sex do coincide. The table constructed for this report was on a weekly basis; that in the submission covering only weeks 40, 46, 52, 60, 68, 76, 84, 88, 92, 96, 100, 104, 108 and 115.

A Life Table Analysis was performed from the Survival Table by Dennis Wilson, Department of Mathematics, Bureau of Foods (Exhibit #73). The female control population differed from the high level population $p < 0.05$. The male control population differed from both the medium and high dose levels ($p = 0.05$ in both cases). In all cases, the differences are due to the higher mortality level of controls.

CLINICAL LABORATORY ANALYSIS

A. Clinical laboratory procedures.

Hematologic, clinical, chemical and urinalysis examinations are described on pages 5-7 of Volume I of the submission. The same rats were employed for all clinical laboratory examinations throughout the study. In cases where one of these rats died during the study, another rat chosen from a corresponding group was substituted.

The following hematology parameters were measured at treatment days 42,92,189,364,547 and 734: hematocrit, hemoglobin, total RBC, total WBC, differential WBC, and prothrombin time.

The following clinical chemistry (serum) measurements were made: pyruvic transaminase (days 42,92,189,364,547,736), glutamic oxaloacetic transaminase (days 41,92,189,364,547 and 734), alkaline phosphatase (days 42,92,189,364,547,734), total bilirubin (days 42,92,189,364,547,734) blood (serum) urea nitrogen (days 42,92,189,364), total cholesterol (days 42,92,189,364,734), L-phenylalanine (days 42,92,189,364,547,734) sodium (day 734), potassium (day 734), calcium (day 734), protein electrophoresis (day 734).

The following urinalysis (2 hour collection) measurements were made at days 42,92,190,364,547, & 734: specific gravity, pH, occult blood, protein, bilirubin, microscopic on sediment, and phenylketones; glucose and ketones were determined at days 42,92,190,364, & 734; urobilinogen was measured at day 42,190,364 and 547.

We noted that some of the data sheets for urinalysis had erroneously labeled the phenylketones test values as "phenylalanine" (see exhibit #84).

Some cholesterol and BUN determinations were carried out which were not described in the submission to FDA. They were as follows:

- 1) Serum cholesterol determinations were done at days 796 & 798 (terminal bleeding), but not included in the submission to FDA.

The protocol indicated that clinical chemistry determinations, including serum cholesterol, were to have been performed at termination. The submission to FDA (Vol. 1 p. 286) reported a significant decrease in serum cholesterol that was more perceptible towards the end of the study, and may have been related to compound administration. Therefore, the omitted data may have been important. (Copies of these data were obtained and are attached as exhibit #77, Section V.

2. BUN determinations were done at day 546 but not reported in the submission to FDA (see exhibit #77 Section V).
 - 3) Serum cholesterols were also done on day 546 and not reported in the submission (see exhibit #77). These determinations were only done for females, and only for a few animals, reportedly due to insufficient quantity of sample.
 - 4) BUN's were also done on day 735 and not reported in the submission. This data was not complete for all animals at day 735.
 - 5) Additional animals (other than those designated) were bled at the regularly scheduled times and determinations were made. These determinations were not reported and we could not determine why the animals were bled. (See Exhibit #77)
- B. A list of persons involved with lab analysis along with their responsibilities and duties is as follows:
- 1) Judith R. Hehmal? - Nov 1971 to present, Supv., Clinical Chemistry, section of Bioanalytical laboratory.
 - 2) Judith A. Beauchamp - Supervisor, Hematology Laboratory, April 1971 to present.
 - 3) Denise Prikins? - Supervisor Hematology Laboratory until April 1971 (no longer employed by Searle).

Bart Tangonan
Tony Martinez
David Kie
Robert Spaet

The above four persons in the toxicology department were involved with assembling data for clinical chemistry and hematology determinations for April 1973 to Feb. 1974.

Joyce Schulmann - performed urinalysis and hematology determinations from April 1973 to Feb. 1974.

Philip Muellner - Technician in Path-Tox Dept. July 1970 till end of study.

Janet Praal - Technician, prepared individual work sheets for urinalysis. No longer employed by Searle.

C. The following employees were interviewed regarding clinical lab procedures, and methods for recording clinical lab. data.

- 1) Bart Tangonan on 6/1/77 regarding the recording of data.
- 2) Judith Beauchamp, on 6/2/77 regarding hematology and urinalysis.
- 3) Judith Schmal, on 6/2/77, 6/7/77, and 7/29/77 regarding clinical chemistry.
- 4) Tony Martinez, on 6/3/77 regarding urine and blood collection, and recording of data.
- 5) Jane Drury, on 6/7/77 regarding electrophoresis.

Accounts of these interviews are attached as exhibits #47-54.

D. Other Documents and Procedures Used to Authenticate Clinical Laboratory Data values in Submission were as follows:

- 1) One loose leaf volume entitled "SC-19192: 104 Week Oral Toxicity Study In The Rat. PT - 988573 Protocols, Organ Weights, Dosage, Hematology, Urinalysis, Blood Chemistry, Protein Electrophoresis." The volume was subdivided into sections according to the above parameters. The individual

pages (See Exhibit #77, Section IX for example) are composed of forms containing the appropriate measurements and units printed on the left side of the page onto which data on "sticky back sheets" corresponding to each of these measurements were pasted in columns representing the various time periods. These pages, in addition to other information, were headed by the identifying number of the rat for which the measurements were made. The information on the sticky back sheets (see Exhibit #77, Sec. IX) was copied (hand written) from laboratory notebooks, sheets, Auto-analyzer Charts, teletype sheets (on line data generated by analytical instruments) or computer printouts (containing raw and calculated data resulting from on line or off line input data from instruments) by individuals of General Toxicology Section (see interview with Bart Tangonan). Many of the pages were initialed "BRT" (apparently by Bart R. Tangonan). Most of the final values transcribed into the sticky back sheets resulted apparently from calculations made directly by the analytical instruments or by external computer using the appropriate stored equations and data for the reference standards.

- (2) Since the values appearing in the volume referred to in the above section were copied from other sources, an attempt was made to verify these values by examining the information in these sources. No attempt was made to recover the teletype sheets, or computer printouts which we were told were no longer available or could not be recovered (see interview with Judith R. Schmal, Exhibit #54). All laboratory notebooks that might contain the original data were requested. Notebooks dated prior to the dates of the DKP study were excluded. The appropriate laboratory notebooks were then identified by BA numbers which were listed on the top sections of the sticky back sheets included in the volume referred to above. Examination of these few laboratory notebooks revealed only a very small amount of data which could be used for additional verification of the values in the submission. It was necessary to obtain the consultation of Judith Schmal to clarify the system used to relate the values in these books with the corresponding rat and period of time of bleeding. The following notebooks, as designated by information on the front covers, were examined:

- 1) Lab. notebook #N-26375 (hematology), 25 June 71 to 1/21/72.
- 2) Lab notebook #127133 (phenylalanine), 10/8/71 to 4/21/72.
- 3) *Lab notebook #113239 (cholesterol), dated 5/1/72.
- 4) *Lab notebook #17, BA #0007118926 (SGOT), 12/27/71 to 2/25/72.
- 5) Lab notebook #126472 (phenylalanine), 4/21/72 to 6/8/72.
- 6) Blue Book #1591, identified "JF VON - 70" (hematology).
- 7) Columnar book #21, identified "JF VON 27" (Differential cell counts).
- 8) Spiral notebook identified "JABEA-" (coagulation/prothrombin) dated 7/23/71.
- 9) *Spiral notebook #16, (SGOT), 8/27/71 to 12/16/71.

*Those books (3,4, & 9 above) marked with an asterisk provided us with no useable data, because a formula or standard curve (no longer available) was necessary to convert the data.

Copies of the applicable pages from all of the above notebooks were obtained, and are included in exhibit #77.

The following data were cross checked against available data from original entries (in addition to being checked against transcribed data on "sticky back sheets" in bound volume):

1. Hematology - Erythrocytes:
Treatment days 42,91,364 & 546 Males and Females.
2. Hematology - Leucocytes, WBC:
Treatment Days 42, 91, 364, & 546 Males and Females.
3. Hematology - Leucocytes, Differential:
Treatment Days 42,91,189,364, & 546, Males and Females.
4. Hematology - Coagulogram, Prothrombin Time:
Treatment Days 42 & 91, Males and Females.

5. Phenylalanine.

Treatment Days 42,189 Males and Females, Day 91 Males.

E. Discrepancies were found between the clinical laboratory methods described on pages 5-7 of submission Volume 1 (referenced on page 120) and those actually carried out. These discrepancies were documented by the interviews described in Section C and in a document (Exhibit #77, Section II) voluntarily submitted by Judith Schmal, June 7, 1977 in response to requests for clarification of the clinical chemistry procedures as they were actually conducted in regard to analytical methodology instrumentation, and processing and recording of data.

1) Glutamic Pyruvic Transaminase.

Reference: Russell, C.D. and Cotlove, E. (1971)/
Clin. Chem. 17; 1114

The reference describes a coupled reaction U.V. assay for serum glutamic oxaloacetic transaminase in which malic dehydrogenase is used.

As described by Judith R. Schmal (June 7, 1977) glutamic pyruvic transaminase was assayed by a method adopted from Sigma Kit Technical bulletin #410 - U. V. using Lactic acid dehydrogenase.

2) Glutamic Oxaloacetic Transaminase

Reference: Same as in (1) above.

As described by Judith R. Schmal (June 7, 1977), from November 1971 to March 15, 1972 a manual colorimetric method (Fermco Kit) was used (employing dinitrophenylhydrazine). From March 15, 1972 the method used was adapted from Sigma Kit, Technical bulletin #410 - U. V. using Lactic acid dehydrogenase.

3) Blood (serum) urea nitrogen.

Reference: Marsh, (Marsh in Submission) W.H., Fingerhut, B. and Miller, H. (1965). Clin Chem 11, 624.

The referenced method calls for reaction of urea with diacetyl monoxime in the presence of thiosemicarbazide and ferric ions in a relatively weak acid solution.

As described by Judith R. Schmal (June 7, 1977) the method used from November 1971 to February 1, 1974 was adapted from Fermco Kit Bulletins #20 and 20-1. Urea is hydrolyzed to ammonia and carbonic acid in the presence of urease. Ammonia is detected by the Berthelot reaction to produce indophenol. From February 1, 1974 the "direct serum method" modified from the method of Marsh et al was used.

4) Phenylalanine

Reference: Hill, J. B., Summer, G. K. Pencer, M. W. Resz N.O. (1965) Chin Chem 11, 541

From Nov. 1971 to about September 1972 there is no documentation in file as to method used. From about September 1972 the method used was a flurometric determination in the presence of ninhydrin and l-leucyl-l-alanine as adapted from McCaman and Rubins. (This is a manual method modified for automation by Hill et al - referenced above) (Judith R. Schmal, June 7, 1977)

5) Calcium:

Reference Pybus J., (Pylrus in submission), Feldman, F. J., and Browsers (Borrers in Submission) Jr., G. N. (1970) Clin. Chem. 16 (11 in submission), 998.

The referenced method involves the measurement of total calcium in serum by atomic absorption spectrophotometry. As described by Judith R. Schmal (June 7, 1977) from November, 1961 to February 1974 the procedure used was a colorimetric procedure using Corinth dye as adapted from Kingsley and Robnet. From May 21, 1973 the method used was atomic absorption spectrophotometry, as adapted from Pybus et all (reference above)

6) Total Cholesterol

Reference: Levine J. S., Morgenstern, S., and Vlastelica, D. (1968). Automation Anal Chem. pp 25-28, Technicon Symposia 1968.

We were unable to check the above reference because of difficulty up to now in obtaining a copy of the publication, but as shown below two different procedures were employed to measure total serum cholesterol at different times during the study.

(Judith R. Schmal, June 7, 1977). From November 1971 to July 2, 1973 the method involved reacting an isopropanol extract of serum with ferric chloride (modified from Block, Tirret and Levine). From July 2, 1973 the method used was a direct serum method using a modified Lieberman - Burchard reaction.

7) Glucose

Reference: Frings, C.S., Ratliff, C.R. and Dunn, R.T. (1969) Advances in Automated Analysis 1, 73

This reference was not checked (because of difficulty up to now in obtaining a copy of the publication) but as shown below two different procedures were employed to measure serum glucose at different times during the study (Judith R. Schmal, June 7, 1977).

From November 1971 to October 16, 1972 the method was a glucose oxidase determination (modified Gertrud Acrow) using protein free filtrates. From October 16, 1972 the method was a direct serum O-Toluidine reaction as modified from Frings, Ratliff and Dunn.

In the case of four of the above parameters (glutamic pyruvic transaminase, glutamic oxaloacetic transaminase, blood urea nitrogen and calcium) different methodology was used during part of the study then was indicated in the submission. For one parameter (phenylalanine), there was no documentation as to the method used for one period of the study and for two other parameters (total cholesterol and glucose), two different methods were used for each of the parameters while only one was referenced in the submission.

Alkaline phosphatase was measured generally as referenced in the submission (McComb, R.B. and Borrers, G.N. (1972). Clin. Chem., 18, 97 in that the method involved measuring the production of p-nitrophenol from p-nitrophenylphosphate. However starting July, 1973 there was a "re-optimization of reagent concentrations" (Judith R. Small, June 7, 1977).

The above changes in procedure could conceivably result in differences in the apparent absolute values for the concentration of the substances measured. Changes in the method of conversion of raw data to calculated values as was done in the determination of sodium and potassium by atomic absorption spectrophotometry during different periods of the study, (Judith R. Schmal, June 7, 1977) could also possibly produce differences in final values.

In an interview with Judith Schmal on June 2, 1977, she did state in response to a question that two levels of "Serum Controls" were used in each run to check the method and instruments and that the data was not reported if the values were more than two standard deviations greater than that for the expected values.

NO evidence was obtained that any attempts were made to determine whether or not DKP could interfere with any of the clinical laboratory tests conducted. For that matter no information was made available to us as to whether DKP itself or related compounds did appear in the blood or the urine of rats fed diet containing this compound.

Neither, as a result of interviews held or reference to available laboratory notebooks were we able to obtain information helpful in explaining the unusually low values for BUN for the control males at treatment days 189 and 364 and for all the treated male groups at treatment day 364. No raw laboratory data in reference to this could be found and may have been recorded on discarded teletype sheets referred to previously. In reference to the low BUN values, Page 29 of the submission contains the following statement: "BUN" values for the control males at treatment day 189 were unusually low and may possibly be related to a technical artifact; as a result, the group mean values for all treated males at this interval were significantly higher but, in fact, these values were in the normal range. BUN values both in control and all treated male groups at treatment day 364 were unusually low; this again reflects a possible technical artifact."

- F. A total of 21 disparities between individual clinical laboratory analysis values appearing in the submission Volume I and those values appearing in data sheets and/or laboratory notebooks were found (Table 4). Of these, 17 were in hematology, one in clinical chemistry, and three in urinalysis. As a result of the discussion with Robert Bost, it was apparent that some of the hematology discrepancies may have resulted for Searle personnel mistaking recorded instrument readings for calculated values. In two cases no value or crossed out values appeared in the laboratory notebooks while values were found entered onto the appropriate places in the data sheets. For animal number A01HM and treatment day 546 four discrepancies (hematocrit, hemoglobin, RBC and WBC) were noted.

G. Discrepancies Found In Statistical Analysis:

The mean and standard errors for the three dose levels and the controls for the various measurements using the values in the submission Volume I or values noted in the data sheets (where there values differed from those found in the submission) were calculated by the Division of Mathematics, FDA. Also supplied were the results of the T-TEsts comparing the controls to the treated groups. See memo to Leonard Friedman from Dennis Wilson, dated July 20, 1977 with attached Tables 1 and 2 (Exhibit #87).

A total of 49 disparities were found, which were comprised of 6 means, 23 standard errors and 20 significant differences. As stated in the memo, in all cases where there is a disparity, it appears to be due to differences in the data.

Calculations were also carried out for cholesterol data found in the data sheets but not reported in the submission. As shown in Table 5 the mean values for the median and high level treated females and the high level treated males were significantly lower than the mean values for the respective controls. To illustrate the possible significance of these changes and disparities between the values calculated by Searle and FDA for cholesterol data at the other time periods of treatment, table 5 was constructed. Very few disparities are seen between the calculated values obtained by FDA and those in the submission but a fairly consistent trend is seen for treatment related lowering of serum cholesterol, particularly at the two highest dose levels and for the female rats.

Because additional disparities were recently noted in individual hematology values after these statistical computations by FDA were completed (due to the discovery of additional laboratory notebooks), and addendum to this report regarding the statistical disparities reported here will be forthcoming.

TABLE 5

TOTAL SERUM CHOLESTEROL (MG/DL)

TREATMENT GROUP	42	92	189	364	734	798
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THIS ENTIRE PAGE OF DATA GUTTED
BEFORE RELEASE OF THIS REPORT
UNDER THE FREEDOM OF INFORMATION ACT!

WHAT DID THE FDA DECIDE TO KEEP FROM
THE PUBLIC IT "PROTECTS"?

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GROSS PATHOLOGY

The pathologists responsible for the microscopic examination (Rudolph Stejskal and Joseph Smith) did not perform the necropsies. Necropsies were performed by Tony Martinez, David Kie and Robert Spaet, with the two pathologists available for consultation.

The submission to FDA (Vol 1, p. 7) reported that "Rats found dead during the study were autopsied immediately whenever possible. In cases where the necropsy could not be performed promptly, the thoracic and abdominal cavities of dead rats were opened and the entire animal was immersed in neutral buffered formalin fixative for subsequent gross examination and dissection".

Our examination of gross pathology records showed that 98 of the 196 animals that died during the study were fixed in toto and autopsied at some later date, in some cases more than one year later.

A total of 20 animals were excluded from the study due to excessive autolysis. Of these, 17 had been fixed in toto and autopsied at a later date. Following are the twenty animals excluded from the study:

Animal No	Date Found Dead	Date Autopsied
C21CM	7/3/73	1/11/74
G16CM	9/21/73	1/11/74
G18CM	8/11/73	10/4/73
G26CM	4/2/73	1/11/74
J2CM	5/21/73	1/11/74
J5CM	10/30/72	11/8/72
L10CM	3/29/73	1/11/74
L15/CM	9/9/73	1/11/74
L21CM	4/13/73	1/11/74
L11LM	5/6/73	1/9/74
A14MM	5/21/73	1/9/74
G28MM	1/5/74	1/7/74
J25MM	5/24/73	5/24/73
A3HN	6/17/73	1/9/74
C15HM	1/7/74	1/7/74

Animal No	Date Found Dead	Date Autopsied
G13HM	7/25/73	**1/9/74
H24CF	4/29/73	1/11/74
D4HF	7/11/73	7/11/73
D16HF	*4/2/73	1/8/74
F6HF	1/5/74	1/7/74

*Although the date found dead was listed as 4/12/73 on the gross pathology sheet, the "Tissue Masses & Deaths" book listed this date as 4/1/73.

**Although the date found dead was listed as 1/9/74 on the gross pathology sheet, the "Tissue Masses & Deaths" book listed this date as 7/25/73.

The gross pathology sheet for one of the above animals, F6HF, described a tissue mass measuring 5.0 X 4.5X2.5 cm. This tissue mass was first observed on 8/24/73 according to the pathology sheet (Exhibit #79), the observation records (Exhibit #70), and the palpation record in the "Tissue Masses and Deaths" book (Exhibit #65). The submission to FDA (Exhibit #8) reported no tissue mass and the animal was excluded from the study due to marked autolysis.

In addition to the above twenty animals that were excluded from the study, many other animals exhibited marked autolysis. For example, D27LF, M25CF, and H12CF are all described grossly in the submission to FDA as follows; "all organs examined grossly were markedly autolyzed".

Records for approximately 30 animals showed substantial differences between gross observations on pathology sheets, when compared with the individual pathology summaries submitted to FDA. Following is a detailed comparison of ten of these. (Copies of all the gross pathology sheets, and the pathology summaries submitted to FDA are attached as Exhibits #78, #79, and #86).

A2CM

Submission to FDA:

Lung	- Focal adhesion
Adrenal	- Moderately enlarged

All other organs examined grossly were unremarkable.

Original Pathology Sheet:

Pituitary - Missing

Lung - Left, mid-portion adheres to the medial area of the rib cage by a "fibrous" type of tissue. (Submitted together with relevant portion of the rib cage).

Right, post-caval lobe has undergone consolidation. Contains grayish-yellowish nodules measuring 2 x 2 mm. (Entire lung submitted in toto)

Lymph Nodes, Pancreatic - Slightly enlarged

Adrenal - Left, moderately enlarged. Right and left, covered with tiny yellow spots measuring 1.0 x 1.0 mm.

Lymph Nodes, Mesenteric - moderately enlarged.

Mass - previously described on 8/20/73 has since then regressed.

Prostate - Marked atrophy, all lobes

Seminal Vesicles - Marked atrophy, bilaterally

All other organs examined were grossly normal and unremarkable.

M15CF

Submission:

Mammary gland - subcutaneous mass located in mid-thoracic region measuring 7 x 6 x 2.5 cm.

Urinary bladder - papillary growth in the lumen.

All other organs examined grossly were unremarkable.

Original:

Mass #1 - Previously described in the left inguinal region on 2/9/73 has since then regressed.

Masses #2 and # - Located in the mid-axillary-cervical regions
are all on mass now measuring 7.0 x 6.0 x 2.5 cm
and may be described as irregular in shape, multi-
nodular, smooth-surfaced, non-glistening,
No Spinal Cord yellowish-purplish in color, non-adherent to the
VL underlying muscle and containing a whitish-yellowish
firm tissue within. (Submitted in toto together with
remainder of tissue).

Heart - Left Ventricle - dilatation and walls thin.
Spleen - Slightly enlarged
Liver - Prominent lobular architecture.
Adrenal - Left, slightly enlarged. Right, unremarkable.
Ovary - Right, small cyst measuring 4.0 x 4.0 mm and
distended with a clear yellow fluid.

All other organs examined were grossly normal and unremarkable.

G10LM

Submission:

Testis - Marked atrophy, unilaterally.
Kidney - Moderate enlargement, mottled appearance, bilaterally.
Small and large intestine exhibited moderate autolysis, no sections
submitted.

All other organs examined were grossly normal and unremarkable.

Original:

Mass which was initially palpated on 2/9/72 (86 days Rx)
in the left inguinal area was actually the left testis which
ascended and went thru weakened left inguinal ring into the
subcutaneous area.

Testis - Left (ascended) appears atrophied (submitted in toto).
Kidney - Moderate, diffuse and uniform enlargement, mottled,
bilaterally (submitted in toto).
Small and large intestines are moderately autolyzed (no sections
submitted).
Thyroid - Moderately enlarged, bilaterally. A 2 mm in dia., dis-
crete, sl raised, moderately firm yellowish-grey lesion
is located in the posterior tip, bilaterally. (Thyroid
submitted in toto wrapped in a lens paper).

All other organs examined were grossly normal and unremarkable.

L11LM

Submission:

Kidney - Mottled appearance
Testes - Marked atrophy, bilaterally
Prostate - Marked atrophy

All other organs examined grossly exhibited marked autolysis.

Original:

Adrenal - Pale yellow, bilaterally
Kidney - Pale yellow, bilaterally, rough-surfaced, bilaterally, moderately autolyzed, bilaterally, tiny spaces in the cortex region measuring about 1 mm in diameter, bilaterally.
Testes - Marked atrophy, bilaterally, marked autolysis, bilaterally.
Prostate - Marked atrophy, all lobes
Seminal Vesicles - Marked atrophy, bilaterally
Spleen - Marked autolysis
Pancreas - Marked autolysis
Stomach - Marked autolysis. A glandular portion - numerous, tiny, pitted ulcerations measuring 1 -4 mm in diameter.
Lymph Nodes, Mesenteric - Marked autolysis
Heart - Wall of left ventricle thin
Brain - Marked autolysis
Pituitary - Marked autolysis
Liver - Marked autolysis

All other organs examined were grossly normal and unremarkable.

M17LF

Submission:

Pituitary - Marked enlargement.
Adrenal - Markedly enlarged and hyperemic, bilaterally.
Mammary Gland - Mass 1, located subcutaneously in left axillary region, measuring 3 X 3 X 2.5 cm; mass 2, located subcutaneously adjacent to mass 1, measuring 3 X 2 X 1 cm; mass 3, located subcutaneously in the right axillary region, measuring 2.5 X 2 X 1 cm; mass 4,

located subcutaneously in the left inguinal region, measuring 3 X 1 X 1 cm; mass 5, located subcutaneously in the right inguinal region, measuring 2 X 1.5 X 1 cm.

All other organs examined grossly were unremarkable.

Original

- Pit - appears markedly hyperemic
- Adrenal - Exhibits numerous minute greyish spots on the serosal surface bilaterally. It appears markedly enlarged.
- Mass (1) - A 3 X 3 X 2.5 cm. spheroidal, multinodular, yellowish white, slightly firm mass located subcutaneously in the left axillary area. Mass non-adherent to the surrounding muscles or tissue (submitted in toto).
- Mass (2) - A 2.5 X 2 X 1 cm spheroidal, smooth, yellowish white firm mass located subcutaneously and adjacent to the above described mass (submitted in toto) mass non-adherent to the surrounding muscles or tissues.
- Mass (3) - A 2.3 X 2 X 1 cm. irregularly shaped, multinodular, yellowish white, firm mass located subcutaneously on the rt. axillary area. Mass non-adherent to the surrounding muscles or tissues (submitted in toto).
- Mass (4) - A 3 X 1 X 1 cm. elongated, multinodular, yellowish white, firm mass located subcutaneously on the left inguinal area. Mass non-adherent to the surrounding muscles or tissues (submitted in toto).
- Mass (5) - A 2 X 1.5 X 1 cm. flat, multinodular, yellowish white, firm mass located subcutaneously on the rt. inguinal area. Mass non-adherent to the surrounding muscles or tissues (submitted in toto).

All other organs examined were grossly normal and unremarkable.

C1MM

Submission

- Kidney Marked enlargement with yellowish discoloration.
- Testis Marked atrophy, bilaterally.

Tissue mass located subcutaneously in the right inguinal area measuring 2.5 X 1 cm.

All other organs examined grossly were unremarkable.

Original:

- Mass - Previously described on 12/9/72 and located subcutaneously in the right inguinal area now measures 2.5 X 2.0 X 1.0 cm and may be described as smooth-surfaced, purplish-yellowish in color, non-glistening, firm, multi-nodular, non-adherent to the underlying muscles and containing a firm yellowish-whitish tissue. (Submitted in toto together with a portion of the skin and underlying muscle with remainder of tissue).
- Heart - Left ventricle has undergone a moderate amount of dilatation. Wall, left ventricle is thin.
- Liver - Prominent lobular architecture.
- Lung - Right, post-caval lobe-consolidation.
- Kidney - Markedly enlarged, yellow and rough-surfaced, bilaterally. Dilatation of the pelvis.
- Adrenal - Covered with tiny yellow spots measuring 1 mm in diameter, bilaterally.
- Testes - Marked atrophy, bilaterally.

All other organs examined were grossly normal and unremarkable.

Tiss. Trimming - Nodules discovered immediately posterior (2.0 cm) to the pyloric portion of the stomach within the adipose tissue. Nodules may be described as firm, yellowish brownish in color. Non-glistening measuring 1.2 X 1.0 mm to 4.0 X 4.0 mm.

E27MM

Submission:

- Lung - Moderate diffuse hyperemia.
- Eye - Opaque cornea, bilaterally.

All other organs examined grossly were unremarkable

Original:

Lungs - All lobes exhibit moderate diffuse and uniform hyperemia.

Kidney - Moderate autolysis.
Eye - The entire cornea is opaque, bilaterally.
Spleen - Moderately autolyzed.
Stomach - Numerous 1-2 mm. hemorrhagic ulcerations are located on the glandular mucosa. Entire small and large intestines are moderately autolyzed.
Brain & Pituitary - Moderately autolyzed.

All other organs examined were grossly normal and unremarkable.

A1HM

Submission:

All organs examined were grossly unremarkable.

Original:

Testes - Markedly atrophy, bilaterally
Lung, Rt - Middle lobe exhibits a 1 X 1 cm consolidation on the posterior portion.
Liver - All lobes appear olive green otherwise unremarkable.

All other organs examined were grossly normal and unremarkable.

L27HM:

Submission:

Testes - Right, slightly enlarged; left, mild atrophy.

All other organs examined grossly were unremarkable.

Original:

Testes - lt./appears markedly atrophy
rt./appears to be distended with yellowish white substance

Seminal V- Appears markedly atrophy bilaterally.
Intestinal - Large, markedly distended with "gas".

All other organs examined were grossly normal and unremarkable.

P.M. Testes - Also, small black areas are noted within along with the yellowish areas. Black areas measuring 1.0 X 1.0 to 4.0 X 4.0 mm in diameter.

J30HM

Submission:

Lung - Moderate consolidation of all right lobes.
Testis - Moderate atrophy

All other organs examined grossly were unremarkable.

Original:

Pituitary - Markedly enlarged; slightly hyperemic.
Heart - Left Ventricle has undergone dilatation walls thin.
Lung - Right, anterior, medial and post-caval lobe have undergone consolidation.
Testes - Marked atrophy, bilaterally.

Seminal Vesicle - Marked atrophy, bilaterally.

All other organs examined were grossly normal and unremarkable.

Dr. Stejskal told us that the other pathologist (Dr. Joseph Smith) who made microscopic evaluations of the slides, came from a hospital background (human pathology) and therefore his descriptions and terminology were a little bit different than one would expect from a veterinary pathologist.

MICROSCOPIC PATHOLOGY

We have assisted in our review of the Microscopic Pathology of Study E-77/78 by Charles H. Frith, D.V.M., Ph.D, Director, Pathology Services, NCTR. Dr. Frith arrived on 6/22/77 and spent 3 days with the FDA team. He examined slides for a representative number of animals, the selection of which was made jointly by Dr. Frith and the other members of the FDA team. A Searle Pathologist was not present during Dr. Frith's review of the slides. However, Dr. Frith did meet with Dr. Rudolf Stejskal, Searle Pathologist, at the conclusion of this review and discussed some of his findings with him.

The first phase of Dr. Frith's review consisted of the examination of the tissues of 25 of the surviving control females and 11 of the non-surviving control females for a total of 36 animals. All of the slides were examined for each animal and the results were compared to the microscopic reports provided by Searle Laboratories. The inconsistencies (findings that differed from those reported by Searle) are listed below:

In most cases the inconsistencies represent findings that were not diagnosed or reported by Searle. Copies of Searle's microscopic pathology reports for each of the animals listed below are attached as exhibit #60.

Female Rat No. F13CF (Path. No. 95617)
Small Intestine - Diverticulum with mucosal necrosis and cellular inflammatory infiltrate.

Female Rat No. F15CF (Path No. 95618)
Pancreas - Focal hyperplasia

Female Rat No. F16CF (Path No. 95619)
Heart - Focal Fibrosis.
Kidney - Mild chronic nephritis.

Female Rat No. H10CF (Path 95624)
Ovary - Neoplasm - probably granulosa cell tumor.

Female Rat No. H19CF (Path. No. 95626)
Kidney - Focal calcification.
Ovary - Neoplasm - probably granulosa cell tumor.

Female Rat No. H30CF (Path. No. 95628)
Kidney - Focal calcification.

Female Rat - No. K25CF (Path No. 95630)
Kidney - Focal calcification.

Female Rat No. K29CF (Path No. 95631)
Heart - Focal fibrosis
Kidney - Focal calcification

Female Rat No. M4CF (Path No. 95632)
Liver - Focal hyperplasia

Female Rat No. M10CF (Path No. 95634)
Kidney - Focal calcification.
Pituitary - Adenoma
Ovary - Fibrosis and Pigmentation.

Female Rat No. M15CF (Path No. 95635)
Pituitary - Adenoma.
Ovary - Cyst.

Female Rat No. B30CF (Path No. 95801)
Kidney - Focal calcification.

Female Rat D29CF (Path No. 95803)
Urinary Bladder (1) Chronic diffuse inflammation.
(2) Diffuse mild hyperplasia.

The second phase of the review consisted of the microscopic examination of all tissues from the high dose females - a total of 36 animals. The inconsistencies are listed below:

Female Rat No. B14HF (Path. No. 95657)
Eye was reported as not examined but eye was present and normal.

Female Rat No. F25HF (Path. No. 95823)
Urinary Bladder - Mild diffuse hyperplasia.

Female Rat No. H7HF (Path No. 95623)
Ovary - Neoplasm - probably granulosa cell tumor.

Female Rat No. H9HF (Path No. 95665)
Heart - Focal fibrosis.
Urinary Bladder - Mild focal hyperplasia.

Female Rat No. H15HF (Path No. 95665)
Lymph Node - The diagnosis of lymphoma, benign, was present on the Searle microscopic report. According to Dr. Frith, lymphoma is generally not considered to be benign and he would diagnose **lymphosarcoma**.

Female Rat NO. H18HF (Path No. 95667)
Pituitary - Adenoma.
Brain - Mild bilateral hydrocephalus.

Female Rat No. K18HF (Path No. 95824)
Pituitary - Adenoma

Female Rat No. K24HF (Path. No. 95671)
Mass noted grossly - nothing consistent with mass reported microscopically.

Female Rat No. - M2HF (Path. No. 95672)
Uterus - Chronic mild endometritis.

Female Rat No. M30HF (Path. No. 95343)
Kidney - Focal calcification.
Uterus - Chronic mild endometritis.

Female Rat No. M30HF (Path. No. 95675)
Pancreas - Focal hyperplasia.

The third phase of this review consisted of microscopic verification of all masses reported grossly at necropsy from all female animals not examined in phases 1 and 2 and included a total of 73 animals. The inconsistencies are listed below:

Female Rat No. D10Lf (Path No. 92521)
Subcutaneous mass was diagnosed as an angiofibroma on Searle report. The lesion is more consistent with an **angiosarcoma**.

Female Rat No. K9MF (Path. No. 95707)
Uterus - Polyp.

Female Rat No. M1LF (Path. No. 95844)
Tissue mass seen grossly was reported as missing and not available for microscopic examination. The tissue was present and was a mammary **fibroadenoma**.

In summary, Dr. Frith reviewed:

- 1) All 36 high dose females (all slides) including 3 that had been excluded from the study due to autolysis.
- 2) 36 (one-half) of the control females (all slides) including 1 animal that had been excluded from the study due to autolysis.
- 3) Remaining 73 female animals with grossly observed masses. (sufficient slides were reviewed to substantiate the masses)
- 4) 5 additional animals selected by the investigators (A1HM, A9HM, A29HM, C2CM, C24HM).

The slides reviewed in the first two categories above constituted 20% of the total animals on the study. Dr. Frith reviewed these slides blindly and then compared his findings with the Searle microscopic reports. According to Dr. Frith, his findings were in agreement with those of Searle, for the most part. In his opinion, some of the lesions that he reported as inconsistencies were small, and might be considered insignificant by some pathologists. Dr. Frith did feel, however, that the **ovarian neoplasms (animals H10CF, H19CF, and H7HP, and chronic cystitis and diffuse hyperplasia (animal D29CF) should have been reported.**

Dr. Frith also considered two other discrepancies to be significant. They were:

- 1) The reporting of a mass (by Searle) as missing which was actually present (M1LF).
- 2) The finding of a polyp of the uterus which was not diagnosed by Searle (K9MF)

The second of the above two discrepancies assumes even more significance in view of the following:

The Histopathologic Summary table (table 11) in Volume I of the submission to FDA lists the following incidence of Uterine Polyps on page 87:

Incidence of Uterine Polyps

Controls	Low	Medium	High
1 of 69 (1%)	1 of 34 (3%)	4 of 34 (12%)	6 of 33 (18%)

The finding of one additional uterine polyp by Dr. Frith (in animal K9MF) increases the incidence in the **mid dose to 5 of 34 (15%).**

On page 82 of Volume I of the submission to FDA, is the statement: "other sporadic findings is included endometrial hyperplasia, polyp, cyst, congestion and squamous metaplasia." **The term "sporadic findings" was used to characterize the incidence of uterine polyps, in spite of the fact that Searle had done a statistical analysis of these findings.**

When this study was reviewed by the Bureau of Foods in 1975, the **dose-related incidence of uterine polyps was noted**. The appropriate slides were requested by FDA at that time and were reviewed by three groups of pathologists: 1.) The Division of Pathology, Bureau of Foods, 2.) Armed Forces Institute of Pathology, 3.) Massachusetts Institute of Technology. Copies of the reports submitted by the 3 groups and related correspondence were obtained and are attached as exhibits #43-45.

Dr. Rudolph Stejskal was responsible for the microscopic findings and accuracy on these findings in the submission to FDA. Only Dr. Stejskal's name appears on the submission. However, a Dr. Joseph H. Smith, M.D. also read slides for this study and his initials appear on some of the microscopic examination sheets. Dr. Frith questioned some of the terminology used in describing tissues. Dr. Stejskal stated that Dr. Smith had come directly to Searle from a hospital situation. Due to his human pathology background, his description of animal tissues was somewhat different than that used by veterinary pathologists.

Dr. Stejskal joined Searle in July of 1973, therefore, he had no input into the pathology protocol, since E-77/78 was initiated in November of 1971.

No microscopic worksheets or other "raw data" relating to microscopic pathology could be found for study E-77/78. We were told by Searle personnel that the original microscopic findings were dictated by the pathologists (Stejskal & Smith) onto belts, and then typed onto sheets which were placed in a binder. The belts were then discarded and apparently the **bound microscopic pathology sheets were either discarded or lost**, after the study report was written. Therefore our verification of the microscopic findings submitted to FDA was limited to a complete inventory of the slides and tissue blocks and microscopic examination of a representative number of slides by Dr. Frith.

Our inventory of the slides and tissue blocks for each animal included a complete list on the tissues sectioned, the number of slides made from each tissue, and a complete count of the total number of slides and blocks for each animal. We also checked the identification numbers on every slide and tissue block. We examined a total of 7,872 slides and 7,360 tissue blocks. The average number of organs submitted for tissue processing was

20 per animal. No errors in slide identification were noted, although in many cases the number of organs submitted for sectioning was less than specified in the protocol. A detailed discussion of this can be found under the heading PROTOCOL.

In addition to the discrepancies noted by Dr. Frith, some other errors were noted in the submission to FDA. A mammary tumor found in rat F27CF was described as a papillary cystadenoma on the individual pathology sheet (page 105, Volume II of the submission to FDA) and as an adenocarcinoma on the summary table 12, page 96, Volume I of the submission to FDA.

Page 92, Volume I of the submission to FDA (a summary table) reports that animal J23CM was found dead after 754 days on study, while the individual pathology sheet for this animal (page 56, Volume II of the submission to FDA) reported that the animal was found dead after 620 days on study. The correct figure is 620 days, since J23CM was placed on the study on 11/17/72 and was found dead on 7/29 /73.

In several instances the histopathology technician made notes at the bottom of the gross pathology sheet to indicate that certain organs were not present in the bottle of fixative. (and were therefore not available for sectioning). Yet in three of these instances (animals A4CM, K23CF, and J3CM) a diagnosis appears in the submission to FDA.

CHARTS, DIAGRAMS AND TABLES

It was necessary to construct a number of charts, diagrams and tables to facilitate our review of the data. For example we constructed a chart, by housing group, showing the identification and complete pathology history of each of the 360 animals. We also rearranged this chart into dosage groups, a copy of which is attached as exhibit #35.

To compare survival data it was necessary to construct a survival table. This also involved devising a calendar to show days and weeks on study for each housing group, taking into account the starting dates for each group. This also included tables showing the numbers of days and weeks animals were on study and a table comparing the survival data from various sources.

We constructed a chart showing diet calculations (gm./kg) and total amounts of DKP used (gm./batch). This is attached as exhibit #30.

Three tables were constructed which summarize the FDA statistical analysis of body/feeder weight data. They are attached as exhibits #39-41.

All of the charts, diagrams and tables that we constructed are attached to the report as exhibits and are referred to in various sections of the report.

EXHIBITS

- #1. G.D. Searle & Company Annual Report for 1976.
- #2. Organizational Chart of Pharmaceutical/Consumer Products Group.
- #3. Organizational Chart of World Wide Pharmaceutical R&D Group.
- #4. Organizational Chart of Preclinical R&D Group.
- #5. Organizational Chart of Product Safety Assessment Group.
- #6. Copies of Computer-Generated Randomization Tables used by Searle to assign the Dose & Housing Groups.
- #7. Diagram showing Typical Housing Group of 30 animals, containing a random distribution of control and treated animals.
- #8. Diagram showing arrangement of food cups on cart, used in feeding the animals.
- #9. Copy of "Glossary of Terms for Aspartame and its Diketopiperazine" and "Analytical Data and Specifications of Food Grade Aspartame".
- #10. Copy of shipping labels for rats received from
- #11. Copy of protocol with amendments for Study P.T. 988S73 (E-77/78).
- #12. Copies of CV's for principal persons involved in study E-77/78.
- #13. Copies of Batch Records for the manufacture of DKP, lots 1R through 5R.
- #14. Copies of pages from Searle chemist Jack Drogt's notebook, concerning the manufacture of DKP.
- #15. Copies of Analytical Reports for DKP, lots 1R through 7R.
- #16. Copy of Searle memorandum dated 12/4/69, concerning DKP Specifications.
- #17. Copy of DKP Specification Sheet (not dated) entitled "Tenative Specifications for SC-19192".

- #18. Copy of DKP Specification Sheet entitled "Specifications for SC-19192, Specification #C40606C".
- #19. Copies of pages 75-84 & 285 of lab notebook #AR-39, concerning assay of DKP, lots 1R, 2R & 3R.
- #20. Copies of pages 60-63 of lab notebook VSH-I, and page 269 of lab notebook book #AR-23, pertaining to analysis of DKP lot 4R.
- #21. Copies of pages 250, 251 and 257 of lab notebook #AR-57, and pages 44-49 of lab notebook #AR-68, pertaining to analysis of DKP lot 5R.
- #22. Copies of pages 83-86 of lab notebook #AR-77, concerning analysis of DKP lot 6R.
- #23. Copy of page 31 of lab notebook #AR-93, concerning analysis of DKP lot 7R.
- #24. Copy of protocol for DKP stability study, dated 1/13/72.
- #25. Copies of pages 51-56 of laboratory notebook #AR-49, assigned to C. Seul. These pages describe a preliminary TLC Test for recovery of DKP from the diet mixture.
- #26. Copies of pages 53-59, 67-72, 88-89, 106-107, 144-145, 156-157, and 284-285 of laboratory notebook #AR-51, assigned to Barbara Bickford. These pages refer to the assay procedure and methods for the DKP Stability Study.
- #27. Copies of Analytical Reports for DKP Stability Study.
- #28. Copies of DKP Compound Inventory Cards.
- #29. Two photographs showing a non-homogeneous sample of DKP diet mixture.
- #30. Chart showing diet calculations (gm.kg.) and total amounts of DKP used (gm./batch).
- #31. Two memos dated 7/14/77 from Thomas F.X. Collins concerning interview with Ray Schroeder.
- #32. Memo dated 7/19/77 from Thomas F. X. Collins describing the 7/18/77 interview with Ray Schroeder.
- #33. Memo of Telephone Conversation between Jerome Bressler and Attorney John H. Bickley Jr., dated July 25, 1977.
- #34. Copies of records concerning calculation of diet concentrations, food concentrations prediction records, dates of bath mixing, and calculation of mean food intake values.
- #35. Charts organized by dose group, showing the identification and pathology history of each of the 360 animals on study.
- #36. Memo dated April 5, 1976, from Dr. John H. Rust to Dr. R. McConnell.
- #37. Searle memo dated September 30, 1974, by Dr. McConnell.

- #38. Memo dated August 29, 1974 from Dr. G. L. Schoenhard to Dr. K.S. Rao.
- #39. Table 1 - Summary of Average Body Weights and Weight gain (%change/week) from the FDA Statistical Analysis.
- #40. Table 2 - Summary of food intake (g/day and g/kg./day and dosage (mg./kg./day) from the FDA Statistical Analysis
- #41. Table 3 - Summary of Food Efficiency (g. gained/100g. actual food consumed) calculated in the FDA Statistical Analysis.
- #42. Computer printout of FDA Statistical Analysis of food intake and body weight data.
- #43. Pathology report from Division of Pathology, Bureau of Foods, concerning uterine polyps, along with correspondence, and memo from Janet Springer.
- #44. Pathology report from Armed Forces Institute of Pathology, concerning uterine polyps.
- #45. Pathology report from Massachusetts Institute of Technology, concerning uterine polyps.
- #46. Written account of interviews with Dr. Jean Taylor on 6/2/77, 6/3/77, and 6/7/77.
- #47. Written account of interview with Judy Beauchamp on 6/2/77.
- #48. Written account of interview with Barbara Bickford on 6/1/77.
- #49. Written account of interview with Clifford J. Seul on 6/2/77.
- #50. Written account of interview with Bartolome R. Tangonan on 6/1/77.
- #51. Written account of interviews with Tony Martinez on 5/19/77, 6/3/77, 7/7/77, and 8/2/77.
- #52. Written account of interview with Ted Reichert on 5/24/77.
- #53. Written account of interview with Barbara Bickford and Clif Seul on 6/2/77.
- #54. Written account of interview with Judith Schmal on 6/2/77 and 6/7/77.
- #55. List of animals bled at 104 and 114 weeks.
- #56. Written account of interview with Alan Mitchell on 7/20/77.
- #57. Written account of interview with Raymond G. Schroeder on 7/18/77.
- #58. Injection records showing administration of penicillin, dates of administration, rat numbers, and units injected.
- #59. Methodology for "Phenistix" determination of phenylketones in urine.

- #60. Dr. Frith's report of examination of slides for DKP study (E-77/78).
- #61. Key for animal identification card numbers used on the body/feeder weight teletype sheets.
- #62. Chart correlating animal cage numbers with pathology numbers, arranged by dose group.
- #63. Chronological list of pathology numbers and corresponding animal cage numbers.
- #64. Organizational charts showing responsibility during the time that study E-77/78 was conducted.
- #65. Volume entitled "tissue masses & deaths". Chronological list of all animals that died, during study, and dates that masses were first observed.
- #66. Charts of days on study for each animal.
- #67. Calendar for duration of study showing starting dates, days and weeks for each group.
- #68. Survival table.
- #69. Charts of Housing/Dosage Groups.
- #70. "Observations for Drug Effects" records for housing groups A through F.
- #71. "Observations for Drug Effects" records for housing groups G through M.
- #72. Ophthalmoscopic records and copies of pathology sheets that have ophthalmoscopic findings.
- #73. Life table analysis and statistics on body weight and food consumption data by Dennis Wilson, Div. of Mathematics, Bureau of Foods.
- #74. Evaluation of feeding study on DKP, a conversion product of Aspartame, by Janet Springer/Ann Ducca, FDA Division on Mathematics.
- #75. Volume A - teletype sheets for body and feeder weight data, housing groups.
 Volume B - "
 Volume C - "
- #76. Copy of Searle Computer Program.
- #77. Volume of protocols, organ weights, dosage, hematology, urinalysis, blood chemistry, and protein electrophoresis.
- #78. Complete gross pathology sheets, males.
- #79. Complete gross pathology sheets, females.
- #80. Key to slide tissue identification numbers and abbreviations.
- #81. Key to stain abbreviations.

- #82. Copies of submission appendix tables relating to hematology, clinical chemistry, urinalysis, and electrophoresis, along with check marks showing errors, and attached copies of raw data sheets documenting the errors.
- #83. Copies of submission appendix tables for organ weights, with errors indicated, and copies of pathology sheets documenting the errors.
- #84. Data sheets showing the phenylketones test erroneously labeled "phenylalanine".

Signed by:

John S. Arnold
Investigator

David M. Eerspamer
Investigator

Dr. Jean Taylor
Toxicologist

Dr. Leonard Friedman
Biochemist

Addendum:

- Exhibit #85 - Copy of Volume 1 of the submission to FDA.
- Exhibit #86 - Copy of Volume 2 of the submission to FDA, consisting of the individual pathology summaries, both gross and microscopic.
- Exhibit #87 - Statistical analysis of Blood and Clinical Chemistry Data by Dennis Wilson, Division of Mathematics, HFF-110.
- Exhibit #88 - Copies of pages from Histology accession book #770C, and Histology inventory sheets.
- Exhibit #89 - Documents from Searle's Math-Stat. Dept. concerning statistical analysis of study 988S73.

Signed

Jerome Bressler
Team Leader

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