(2)

Dec. #50

ESTABLISHMENT INSPECTION ENDORSEMENT

Page of Pages

1. ESTABLISHMENT

a. ESTABLISHMENT NAME
Searle Laboratories
Div. of G.D. Searle & Co.

b. DISTRICT
Chicago

c. CENTRAL FILE NO

d. ESTABLISHMENT ADDRESS (Include Zip Code, Area Code and Telephone No.)
4901 Searle Parkway
Skokie, IL 60676

e. DATE INSPECTED
4/25/77-8/4/

2. ROUTING

a. HEADQUARTERS UNIT TO WHICH REFERRED (Use organizational symbol)
Bureau of Foods, HFF-330
Attn: Mr. Richard Ronk

Recorded
CHICAGO

b. REASON FOR REFERRAL
To Be Reviewed by the Bureau of Foods

HEADQUARTERS USE ONLY

c. DATE REFERRED
PROFILE NOT NEEDED
BY NH DATE 8-1

d. AF NUMBER

3. DISTRICT REMARKS

a. DISTRICT ENDORSEMENT

This top priority investigation was made to compare all available raw and summary data, along with all related material including methodologies, against the FDA submission. This inspection covers one study.

E-77/78 (P.T. 988573), SC-19192: 115 Week Oral Tumorigenicity Study in the Rat - Diketopiperazine

Study E-77/78 was initiated on November 8, 1971. The FDA submission is dated September 1974.

Three hundred and sixty weanling albino rats, Charles River CD strain, 180 of each sex, were used. The rats were divided into twelve housing groups, (six groups per sex), thirty rats in each housing group. Each housing group was composed of a random distribution of Control, Low, Mid, and High Dose animals. The rats were fed Diketopiperizine (SC-19192) at 0, 0.75, 1.5, and 3.0 grams per kilogram of body weight per day respectively

Our investigation of this study shows that no homogeneity tests were performed on any batches of the diet. We found evidence that the diets were not homogeneous.

Two unidentified infectious disease outbreaks were reported in the FDA submission. In both instances the control and treated animals were reportedly affected with equal frequency and severity. All morbid rats were injected with potassium penicillin G. Our review of the records show a third occurrence of infectious disease and penicillin administration took place, which was not reported in the submission to FDA.

We found an additional polyp of the uterus in the mid dose group which was not diagnosed or reported in the submission

b. REVIEWING OFFICER (Name and title)
Jerome Bressler

c. SIGNATURE
Jerome Bressler

d. DATE
8/7/77

e. DISTRIBUTION
(C: CHI-DO) CC: HFF-330, HFF-1, HFO-1, HFA-224, HFR-5140

FORM FD 766 (11/75) PREVIOUS EDITION MAY BE USED UNTIL SUPPLY IS EXHAUSTED.

by Searle. The finding of one additional uterine polyp increases the incidence in the mid dose to 5 polyps of 34 animals (15%). The incidence of polyps of the uterus appears to be dose related.

Serum cholesterol determinations were done at days 796 and 798 (terminal bleeding), but not included in the submission to FDA. The submission reported a significant decrease in serum cholesterol that was more perceptible toward the end of the study and may have been dose related. Therefore, the exclusion of data from days 796 and 798 could be significant.

In some instances raw data was not available for review especially in the areas of clinical chemistry and microscopic pathology. In other instances there were inconsistencies in the raw data making it difficult to authenticate the study. As the investigation proceeded we learned that not all of the data was under seal. We discovered a number of documents. It is quite probable that there are still some laboratory notebooks missing. The majority of the responsible individuals that worked on this study are no longer with Searle.

FOLLOW-UP: To be reviewed by the Bureau of Foods

4/25/77 to 8/4/77

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Searle Laboratories
Div. G.D. Searle & Co.
4901 Searle Parkway
Skokie, Illinois 60076

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 PT 988S73, SC-19192: 115 Week Oral Tumorigenicity Study in the Rat, are as follows:

A. Design & 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 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 infectious 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 Mixture

1) 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.

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 two 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 study employed Basal Diet from week 62 to its conclusion, and no 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 record A-9129 for DKP lot 5R showed an assay of 100.0%. 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., Director, 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 MILF).

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 mid dose to 5 of 34. (15 percent).

c. The finding of ovarian neoplasms in animals H10CF, H19CF, 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. 96, 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 male control population differed from the mid and high level population (p 0.05). In all cases the differences are due to the higher mortality in the controls.

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Div. G.D. Searle & Co.
Skokie, Illinois 60076

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 submission Methods or Results section but 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 initialled 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 intial the data sheets.

5. The methodolgy 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".

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Div. G.D. Searle & Co.
Skokie, Illinois 60076

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 sweetner 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 Embryotoxic and Teratogenic Potential in the Rat, conducted with SC-13862 (aspartame).

2.) E-89 (PT #1218S75), An Evaluation of the Embryotoxic and Teratogenic 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 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 4-25-77.

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. Seul - 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).

Searle Laboratories
Div. G.D. Searle & Co.
Skokie, Illinois 60076

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-18852) 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

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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 SPECIFIED IN PROTOCOL	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