DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE
PUBLIC HEALTH SERVICE
FOOD AND DRUG ADMINISTRATION

September 26, 1979

CHICAGO DISTRICT
1222—POST OFFICE BUILDING
433 WEST VAN BUREN STREET
CHICAGO, ILLINOIS 60607
TELEPHONE: 312-353-5863

John W. Olney, M. D.
Department of Psychiatry
Barnes and Renard Hospitals
4940 Audubon Avenue
St. Louis, Missouri 63110

Re: FOI # 79-21004
CHI- 1766

Dear Dr. Olney:

This is in response to your request of August 20, 1979 for records from the Food and Drug Administration pursuant to the Freedom of Information Act: Paragraph III of your letter re: G. D. Searle's product: Aspartame.

X We are enclosing the requested record(s) consisting of EIR dated 4/25 - 8/4/77 regarding study of E 77/78 and EIR dated 5/2 - 7/8/77 regarding study E-5 and E89.

X As you will note, minor deletions of material have been made in the records furnished to you. In the judgment of the Food and Drug Administration, the information deleted does not fall within the scope of your request and, in any case, is not required to be disclosed under the Freedom of Information Act. If, however, you do desire to review the deleted material, please make an additional request. If the agency should then deny you this information, you would have the right to appeal such denial to the Department of Health, Education, and Welfare. Any letter of denial will tell you how to make this appeal.

____ No deletions were made from this material.

____ The requested record(s) will be sent at a later date.

X I apologize for the quality of the 5/2 - 7/77 EIR. The problems were with our original copy. Please contact me at 312-353-5863 if you have problems.

X We are assessing the following charges:

125 pages	@ $ .10 =	$12.50
2 hours search time -1/2 hour	@ 3.00 =	4.50
Total		$17.00

An invoice is attached.

____ The charges will be aggregated to your bill.

____ There will be no charge for furnishing record(s).

Sincerely,

George F. Bailey

George F. Bailey
Freedom of Information Officer

Enclosure: a/s
GFB/ed

EIR 5/2-7/8/77
CEL, JFS, TC

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SUMMARY OF FINDINGS

We made a detailed inspection of the raw data versus the final report on two teratology studies on SC 18862 (aspartame). These studies, numbered E-5 (PT851S70) and E-89 (PT1218S75) were selected for our inspectional coverage by headquarters personnel of the Bureau of Foods. Study number E-5, "SC-18862: Evaluation of Embryotoxic and Teratogenic Potential in the Rat" had not been previously inspected by FDA personnel at Searle Laboratories. Study number E-89 was included as one of five teratology/reproduction studies that were covered by an FDA inspection team during the period of December 1 through 19, 1975.

Our inspection of Study E-5 included the following findings:

1. The individual doing the examinations of the visceral and skeleton specimens was aware of the dose levels. The examinations were not done blind.

2. There are no individual fetus records for the skeletal examinations. The skeletal examination data is listed only by litter under the dam number. The skeletal examination records are not dated.

3. There are no examination sheets that specify the abnormalities that are included in their examination of visceral sections. Their visceral examination records indicate only "O.K." if no abnormalities were found. The visceral examination sheets do not list the respective fetus identification numbers for about 10% of the 329 fetus visceral specimens. These incompletely identified fetus specimens are identified on the examination sheet with only the dam number and fetus sex.

4. According to the visceral examination records, a total of 329 visceral examinations might have been done on two days. We were unable to examine any visceral sections from study E-5 because they had been discarded.

5. There were no signatures or initials to identify the individuals who did the work on the skeletal, visceral, and laporotomy examination sheets.

6. There was no identification on the body of the vials holding the skeleton specimens; the respective fetus number was on the vial cap (See exhibit 39, photo 1).

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7. There was no record to verify the source and age of the male rats.

8. There were no specifications or assay records on the basal diet.

9. There were no batch records for the mixing of the powdered SC 18862 (aspartame) with the meal form of Rockland diet (Teklad Inc.) Mrs. D. Helms, Research Assistant, could not remember the details of mixing - such as the total mixing time or the order of adding the SC 18862 and the Rockland Diet to the mixer.

10. The treatment mixtures (two dose levels) were not assayed for potency, homogeneity or stability.

11. The examination of the fetal skeletons of 5 litters of each dose level by Dr. T. Collins revealed only a few differences from their original skeletal examination data as compared to the FDA submission. A few differences in the results are not unusual between 2 individuals when they are doing examinations. These findings are detailed in the body of the report.

Our inspection of study number E-89 included the following findings:

1. The individual who did the visceral and skeleton examination was aware of the dose level of the specimens that were being examined.

2. There are no examination sheets that specify the abnormalities that are included in Searle's examination of visceral sections.

3. The only identification of the skeleton specimens is on the caps of the vials with the respective fetus number and the PT number, 1218.

4. The records covered receipt of only 10 of the 36 male ~~rats~~.

5. There were no signatures or initials to identify the individuals who did the work on the skeleton examination records.

6. There were no assay reports or specifications on the basal diet.

7. There were no batch records for the mixing of the aspartame with the chow. The three treatment mixtures were not assayed for potency, homogeneity or stability.

8. Searle did not include any abnormal findings of visceral examination in the report that was submitted to FDA. The raw

data included major malformations of a segmented uterus in a low dose fetus 20407 and a cleft palate in a medium dose fetus 32012 neither of which was included in the FDA submission. Dr. Vondruska was shown this data and said this omission was an oversight (see Vondruska's interview). Dr. Collins examined visceral sections that included verification of the aforementioned findings. Dr. Collins also noted a slight hydrocephalus of fetus 20407, low dose, that was not in the raw data or the FDA submission. This was confirmed by Dr. J. Noverske the Searle Teratologist. (see exhibit 39, photo 2) Dr. Collins disagreed with Searle's classification of "renal pelvic cavitation of the kidney not enlarged" of the fetus 41101 as an artifact and not a malformation. (see exhibit 39, photo 4) Dr. Collins does not agree that this is an artifact and he is of the opinion that it is due to the blockage of the urinary tract.

Dr. Vondruska stated that in retrospect "artifact" was probably a poor word to use. He said that Coll might have sectioned the kidneys at an incorrect angle, thereby, giving the appearance of an enlarged renal pelvis. (see Vondruska interview)

9. It would appear that the visceral sections were cut too thick. There would be a possibility that some visceral abnormalities would be missed.

10. It was noted in the FDA submission that there was a significantly greater number of fetuses in the medium dose level with poorly ossified supraoccipital bones, when compared to the control group. Because of this finding, the supraoccipital bones of the fetuses in the high dose level were examined. Dr. Collins scanned the supraoccipital bone for poor ossification in each of the skeletal fetuses of the control and high dosage groups. His examination of the supraoccipital bone revealed the following percentage differences from the FDA submission.

Supraoccipital Bone Poorly Ossified	Control Fetuses	High Dose Fetuses
FDA Submission	3%	6%
Examination by Dr. T. Collins	4.46%	8.47%

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11. C. Kirby, Reasearch Technician whose duties included the visceral and skeletal fetal examinations and laparotomies for study E-89 completed about three years of college. She started employment with Searle Laboratories in August of 1974 and performed visceral and skeletal exams on E-89 in May and June of 1975. This was the only study where she performed the visceral exams. She stated that her on-the-job training consisted of a total of about 3 months.

12. There are no dates of examination on the skeleton tables (exhibit 30). On the back of the laparotomy sheets, the major skeletal variations are listed. Most of the skeletal examinations are dated 5/19/75 and 6/4/75. It would be impossible for one individual to do a complete skeletal examination of over 500 fetuses in 2 days. It is unclear over what period of time these fetuses were read.

PURPOSE OF INVESTIGATION

Assignment memo dated May 16, 1977 from Donald Healton, Acting Executive 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 investigating began on 4/25/77 (see EIR E 77/78) and encompassed the authentication of all data, both raw and summary, relating to the studies jointly chosen for review by the 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.

We began our investigation of E-5 (PT-851S70) Evaluations of Embryotoxic and Teratogenic Potential in the rat, using SC18862 (Aspartame), on May 2, 1977.

On May 11, 1977, after clearance from the Bureau of Foods, we initiated the investigation of E-89 (PT-1218S75) an Evaluation of Embryotoxic and Teratogenic Potential in the mouse, using SC-18862 (Aspartame), see assignment attached.

This report is concerned with the above two studies. The report involving E-77/78 will be reported separately.

REFUSALS

Attached as exhibit 40 is a memorandum dated June 29, 1977 from Mr. Roger Thies, Attorney refusing our request for an additional interview of Ms. Gail Kirby, a technician who worked on E-89 (PT-1218S75), Evaluation of Embryotoxic and Teratogenic Potential in the mouse (aspartame).

We were concerned with the dates shown on the back of the laparotomy sheets, "6/14/75" and "5/19/75." Dr. Collins is of the opinion that it would be extremely difficult to completely examine 300 skeletons in two days, if these dates, so indicate. In our interview with Mr. Schroeder, a former employee, he told us that he was able to examine thirty skeletons in a day. (see Schoeder interview attached) In our interview with Dr. Vondruska, he could not not explain the dates shown on the back of the laparotomy sheets. He told Dr. Collins that he would have to ask Ms. Gail Kirby.

Our failure to interview Ms. Gail Kirby leaves the question of the dates unresolved. G.D. Searle's refusal to allow us to conduct a telephone interview is given in the menorandum from Mr. Thies (see exhibit 40). We do not consider his reasons for refusal as valid.

PERSONS INTERVIEWED

Investigators Carl E. Lorentzson and Johnny F. Salas presented their credentials and issued a Notice of Inspection on May 2, 1977 to Richard E. Viktora, Attorney. Dr. Thomas F.X. Collins issued a Notice of Inspection on May 4, 1977 to Dr. William M. Merino, Director of Regulatory Affairs. Dr. Collins was at Searle Laboratories on May 4-6, 23-27, June 6-7, and July 7 and 8, 1977. Investigators Carl E. Lorentzson and/or Johnny F. Salas were both present on each date of inspection with the exception of July 7 and 8, 1977 Investigator J. Salas was present at Searle Laboratories for the inspection of studies E-89 and E-5 on May 17, 1977, when Investigator C. Lorentzson was not at Searle Laboratories. An attorney and/or a Ph.D. from one of the research units of Searle Laboratories was present whenever we reviewed records, inspected the facilities, examined

fetal skeletons or interviewed personnel. These individuals were:

Dr. Robert Post - Director of Food Products, Regulatory Affairs
Dr. George Clay - CNS Group Leader
Richard Viktora - Attorney
Roger Thies - Attorney
Dr. J. Neveroske - Group Leader of Toxicology
Dr. Fred M. Radzialowski - Section Leader of Cardio-vascular Pharmacology
Dr. K. Jenkins - Director of Product Affairs
Dr. Richard L. Aspinall - Group Leader of Immunology & Inflammatory Diseases

We interviewed Research Assistant Mrs. D. Helms at Searle Laboratories regarding her duties on study E-5.

We made arrangements to interview Raymond Schroeder, a former employee whose title at the time was Senior Research Assistant, and whose principal duties were on study E-5 and relatively limited duties on study E-99. This interivew was conducted in New Jersey because Raymond Schroeder is now residing in Somerville, N.J.

We interviewed the following individuals regarding their duties on study E-99:

1. Gail Kirby - Research Technician
2. Jeanne Thompson - Research Technician
3. Dr. J.F. Vondruska - Senior Investigator
4. Alan Mitchell - Teratologist

Richard Viktora provided us with the date that Raymond D. Schroeder left this firm, namely May 2, 1975. However, Mr. Viktora said that he would not furnish a copy of a record to substantiate this termination date because it would be a violation of the Equal Employment Opportunity Regulations. We were allowed to review and make notes from the following records. However, Roger Thies, Attorney, did not allow photocopies because he did not consider these records to be primary data on study E-5, namely:

1. A preliminary draft of the summary and conclusions for the final report on "NF 851570" (Searle Doc #1144652)

2. A list of the studies which either have been completed or were in progress with aspartame to determine the relative toxicity of aspartame and Diketopiperazine in several species of animals. (Searle Doc #1272358)

3. A "galley copy" of the report that was submitted to FDA

4. An inventory list of the teratology specimens that were stored in a basement storage area. The record included a listing of the fetal skeleton preparation from the rat, in study E-5 (PT-851S70) in box numbers T-043A, T015.

SCOPE OF OUR INSPECTION

We requested all of the records pertaining to study E-5 on the first day of our inspection, May 2, 1977. It was brought to our attention by Jerome Bressler, FDA inspection team leader, that the data pertaining to this teratology study had been previously placed under FDA seal. We then visited their R&D central file room to locate these records. We determined that the data including primary records pertaining to their teratology studies on SC 18862 (aspartame) was stored under FDA seal in two file drawers. We initially attempted to remove the data from these file drawers that pertained only to study E-5. In order to facilitate our detailed examination of these records on teratology studies, we then removed the records on all of the teratology studies in their two file drawers to a room on the first floor of "J" building. Whenever we did not personally guard these records, we maintained the data on these teratology studies in a locked metal cabinet under FDA seal. We obtained almost all of the records for our E-5 study from their central file room. We subsequently requested additional records pertaining to the study E-5 such as the lab testing of the component, SC 18862; invoice for purchase of female rats; curriculum vitae and chain of responsibility. We made photocopies of essentially all primary data and other records pertaining to study E-5. Exhibit numbers 1 through 13, 38 and 3 photos in Exhibit 39 pertain to study E-5.

We made a detailed review of all raw data against the report that was submitted to FDA. This review included fetal and maternal body weights, maternal food consumption, crown rump measurements, number of corpora lutea, number of live and dead fetuses and examination records on visceral and skeletal fetal specimens. Dr. T. Collins examined skeletal specimens from study E-5, and skeletal and visceral specimens from study E-89.

After we completed the majority of our inspectional work at Searle Laboratories on study E-5, we received authorization from personnel

of the Bureau of Foods on May 11, 1977 to institute an inspection of an additional teratology study, E-89 (PT-1218875) entitled - "SC-18862 - An Evlauation of the Embryotoxic and Teratogenic Potential in the Mouse". We made copies of all primary data and other records pertaining to study E-89. Exhibit numbers 16 through 38 and photo numbers 2, 3, and 4 of Exhibit 39 pertain to study E-89. We made a comprehensive review of all raw data with one minor exception. We estimate that we checked more than one third of the food consumption primary data for accuracy. The previous inspection of December 1-19, 1975 included study E-89 and stated in part that maternal food consumption was transferred without error from the raw data. The FDA submission on study E-89 states in part that the pregnant animals actually consumed dose levels for the low, medium, and high dose groups respectively which are approximately 40% more than the originally intended doses of 1.0, 2.0 and 4.0 g/Kg.

PERSONNEL ON THE E-5 STUDY

Individual	Title & Background	Duties
Mrs. Donna Helms	Research Assistant Her educational background includes B.S. Univ. of Wisconsin with a major in Zoology in 1966. She started work for Searle Laboratories in 1969 and is currently employed by the firm.	Donna Helms stated that her duties included: weighing of the animals; setting up the study; food consumption data; transfer of data from cage cards to laporotomy sheets; and performing hysterotomies.
Raymond F. Schroeder	Senior Research Assistant in Teratology. His education includes a M.S. in Zoology from the Univ. of Illinois in 1967. He was employed by Searle Laboratories from Dec., 1967 to May 2, 1975.	According to Donna Helms, the duties of Ray Schroeder included external observation of the fetuses; supervision of the laporotomy; and performance of the visceral sections and skeletal examinations.

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Margaret S. Faber (Hoppenrath)	Bio Research Technician	Donna Helms stated that Margaret Faber might have done some of the crown rump measurements. Donna Helms was unable to recall any other work that was done by Margaret Faber on study #L-5. Raymond Schroeder informed us during his interview that the duties of Margaret Faber (Hoppenrath) included: killing of animals, mixing of the diet, crown-rump measurements, weighing of fetuses, staining of skeletons, and cutting the visceral sections.

Copies of Curriculum vitae for key personnel and a listing of the responsible individuals of Searle Laboratories during the years 1969 and 1970 are attached as exhibits numbered 1 and 2. Study number L-5 was conducted during the first half of 1970. The Director of Biology of Searle Laboratories during this time period was V.A. Drill. The authors of the report are R.E. Schroeder and R.C. McConnell, Dept. of Pathology-Toxicology, Division of Biological Research.

Study L-5 (PT 851570)

SC-16662: Evaluation of the Embryotoxic and Teratogenic Potential in the Rat

Date study initiated: Jan. 20, 1970

Dates of performing laparotomies: Feb. 9 through Feb. 19, 1970

Date study was received by Bureau of Foods: August 7, 1972

Animals:

Species and Strain - Albino rat, Charles River caesarian derived virgin females and proven males

Number and Sex - 90 females, 30 males - there were no records to indicate source and age of male rats. He verified that the females were approximately 100 days old at time of mating - Invoice (Exhibit 4) indicates date of receipt: 12/30/69.

Experimental Design:

Ninety females were distributed into the following three groups. Mrs. D. Helms said that she used a randomization method that involved drawing animal numbers from pieces of paper in a hat. She didn't remember if the first number drawn was assigned to a control group.

Dosage Group	No of animals	Dose Level - mg/kg
Control	30	0
Low	30	2000
High	30	4000

The respective identification number of each of the rats was punch marked on their ears.

Donna Helms could not state definitely whether the animals from each dose group had a unique color marking on their tail. Three females, one from each of three dosage groups were housed together in a breeding cage. At 4:30 p.m. one male was placed into each cage; he was removed at 8:30 a.m. the following morning. At that time females were examined for a copulatory vaginal plug and/or spermatozoa in the vaginal smear. Observation of either of these signs indicated mating and was designated day 0 of pregnancy. Such females were removed from the breeding cage and housed individually. They put this rat in the next empty cage going from left to right. This procedure was continued until a minimum of 24 females from each group were mated. Copies of the cage identification cards are attached as Exhibit number 6. We were informed that any daily observations would be recorded on these cage cards. There are no records of abnormal observations on these cards.

Dietary administration of SC-18862 at the dose indicated (2.34% and 5.00% concentration respectively) began on day 6 of gestation and continued through day 15 of gestation, a 10 day period of treatment. The females were sacrificed on day 20 of gestation. The uterine horns were exposed and examined. The fetuses were removed, examined externally and preserved intact to be examined later for visceral irregularities (Wilson Technique) or skeleton anomalies (Alizarin Red S Skeletal staining technique).

Dose Group	Bred	Surviving	Pregnant
Control	27	27	26
Low	25	25	24
High	24	24	23

Donna Helms could not remember the exact animal room in which this experiment took place. However, she showed us an animal room that closely resembled the actual room that was used to house the animals for study E-5. This room had only one doorway that was used for both the entrance and exit. The room had equipment to control the temperature and adjust the number of hours of light and darkness.

A photocopy of their protocol is attached as Exhibit 3. Our review of this protocol reveals that it is essentially in conformance with their FDA submission on study E-5 (Exhibit 13).

Formulation of SC 18862

The SC 18862 was mixed with the basal diet in weight per weight concentrations of 2.34% and 5.0% respectively for the low and high dose groups.

The [redacted] Model, V-1401 mixer that was used during 1970 in the research facility of Searle Laboratories in Skokie, Illinois was subsequently moved to another division of the G.D. Searle Company. This mixer was then returned to Searle Laboratories in Skokie, Ill. where it is currently being used in their Pharmaceutical Development area. We inspected this mixer (about 5 feet high) and noted that it was currently being used with a mixing bowl that would hold approximately 20 kilos of a treatment mixture. The treatment

page 6 of the FDA submission is essentially correct. The amount of SC-16862 actually consumed closely approximated the planned dosages of 2000 and 4000 mg/Kg. On the basis of mean body weights on days 6 and 11 of gestation and mean food consumption from gestation days 6-15, the actual daily doses consumed by the low and high dose groups were 1,955 and 4,094 mg/Kg body weight respectively. Our calculations of the food consumption data revealed results that are within 1% of these reported average daily doses.

Hysterectomy Data

Donna Helms said that their hysterectomies were usually done in the morning. Their original records do not indicate observations of any lesions of the ovaries or uterus in any of the animals at sacrifice. The series of numbered hysterotomy sheets includes missing consecutive number hysterotomy sheets for animals that never mated. Our comparison of their original hysterotomy data (Exhibits 6, 9, & 10) and the tables numbered 1, 2, 3, & 4 in the FDA submission revealed only a few discrepancies. These hysterotomy tables included data i.e.: number of live and dead fetuses; sex of fetuses; number of resorptions; average fetal weight; and average crown rump measurements.

We noted the following discrepancies:

1. Table 2 of the FDA submission indicates that the average fetal weight for animal 29 of the control group is 4.0 grams; the average fetal weight for this animal is actually 3.9.

2. Original hysterotomy records indicate that there was one resorption on the "left" side for animal number 11 of the control group; table 2 of the FDA submission does not list this resorption on the left side. The FDA submission correctly lists the two resorptions that are marked on the right side of animal number 11 on their laparotomy sheet. Mr. R. Schroeder acknowledged these errors. (see R. Schroeder interview)

3. We noted the listing of one resorption for animal 72 on the laparotomy sheet; this resorption is not listed in the FDA submission. Mr. R. Schroeder acknowledged this omission and said it might have been a typographical error.

mixture batch sizes for study S-5 were 3 kilos or smaller. It was brought to our attention that this mixer was formerly equipped with a smaller mixing bowl and a smaller anchor shaped mixing blade when it was used to mix treatment mixtures of aspartame in 1970. During our interview with R. Schroeder, he described a smaller mixer (about 2 feet high) with a 10-gallon capacity mixing bowl.

Searle did not maintain batch records for the mixing of powdered SC-18862 with the meal form of Rockland Diet ( ) There were no specifications or assay records on the Rockland Diet. We were informed that the manufacturer of the Basal Diet is out of business. Lot number 74020 of SC-18862, a white powder, obtained from the manufacturer was incorporated in the treatment mixtures. This material was submitted to lab testing that included: identity tests; pH in water; melting range; specific rotation; total nitrogen content; loss on drying; heavy metals and thin layer chromatography. Copies of their records regarding lab testing of this lot of SC-18862 are attached as Exhibit 5.

The treatment mixtures (two dose levels) were not assayed for potency, homogeneity or stability. The treatment mixtures were mixed in the mixer in the "Diet Kitchen" by Raymond Schroeder, Senior Research Assistant in Teratology or Mrs. Donna Helms, Research Assistant. Mrs. D. Helms could not remember the details of mixing such as the order of adding the SC-18862 and the Rockland Diet to the Hobart mixer. Reserve samples of the treatment mixtures were not maintained. Additional details regarding the treatment mixtures are included under a subsequent heading of "Interview of Raymond Schroeder."

Food Consumption

Copies of the food consumption records are attached as Exhibit 7. A quantity of food consumption that is underlined on these records indicates that a weighed quantity of spillage has been subtracted. Donna Helms said that food consumption was always measured first thing in the morning. Donna explained that in an attempt to account for food spillage she separated the food from the excreta on the tray beneath the respective animal cage. Donna Helms said that she covered the feed jars with a V-type mesh screen for the rats that were considered "chronic spillers". She said that the feed was transferred to smaller size jars during the course of the study in order to make it easier for the pregnant rats to reach their food. Our calculation of their food consumption records indicated that their statement regarding food consumption on

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Skeletal Examinations - B-5

There are no individual fetus records for the skeletal examinations. The skeletal examination data is listed only by litter under the respective "Dam number". The skeleton examination records were not dated and did not bear any signatures or initials. Mr. E. Schroeder was shown these records and stated that they should have been dated. He said that it took a great deal of time to complete the skeletal readings. He also stated that it took him 5-6 minutes to do a complete skeletal examination of one fetus. (see interview with Mr. Schroeder) We compared the original skeletal examination records (Exhibit 11) with the report that was submitted to FDA (Exhibits 1-13). Dr. T. Collins also examined skeletal specimens of 5 litters of each dose level.

We noted the following:

1. The original skeletal examination records indicate a finding of "Hypoplasia of the Maxilla" in one fetus of Dam 57 and one fetus of Dam 58; (see exhibit 39, photo 6), this finding is not in the FDA submission. Mr. E. Schroeder acknowledged these errors.

2. The original skeletal examination records list a total of 166 (83%) fetal skeletons with unossified cervical centrum in the control group. The original records do not indicate how many of the cervical vertebrae had less than 3 ossified centra. The FDA submission indicates a total of 83 control fetuses had unossified cervical centrum with less than 3 centra ossified. It is probable that an error was made in transcribing the percentage of 83 instead of the total of 166 fetal skeletons with unossified cervica centrual to the FDA submission.

3. The original skeletal exes records indicate 3% upper and 1% lower incisors absent for the control group, 4% upper and 4% lower incisors absent for the low dose group and 5% upper incisors absent for the high dose. These are not mentioned in the FDA submission.

4. The original skeletal exes records indicate one sternum ossification center split for the control dose group; this sternum ossification split is not listed in the FDA submission.

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5. The rudimentary structures are small projections from the first lumbar vertebrae. These are small 14th ribs. Most animals with these structures are graded twice. They are counted as having 13 pairs of ribs as well as rudimentary structures.

6. Dr. T. Collins' examination of fetal skeletal specimens of 5 litters of each dose level revealed only a few differences from what was contained in the raw data that would alter the conclusion of the study.

7. Dr. Collins stated in effect that it would have been a better procedure to grade individual bones instead of closure grading for skeletal examination of the skull. (see Exhibit 11)

8. We made a physical inventory of the skeletal specimens. We compared this inventory against the skeletal fetal specimens that are designated on the laparotomy records (Exhibit 8, 9, & 10) as "A" for fetuses that were supposed to be initially preserved in 95% alcohol prior to staining, evisceration, clearing with aqueous potassium hydroxide, staining with Alizarin Red and storage of the skeletal preparation in glycerin. This inventory revealed that a total of 15 skeletal fetuses from the high dose group were missing. We were unable to obtain a definite reason as to why the following fetal skeletons were not in inventory: 6902, 6405, 8612, 8908, 8909, 8911, 8913, 9002, 9003, 9005, 9006, 9008, 9009, 9011, and 9013. Dr. J. Noveroske, Group Leader in Toxicology speculated that the four skeletal specimens from litter number 89 and the skeletal specimens from litter number 90 might be in a separate carton that was inadvertently displaced.

9. Dr. T. Collins found mistakes in examining the skeletal specimens of all dose levels. As an example Dr. T. Collins noted a poorly ossified ischium for a fetus of Dam 58; this finding is not in the FDA submission. (see exhibit 39, photo 5) These mistakes appear to be equally distributed between the dose levels. Searle's examination of the skeletal specimens corresponds essentially with the FDA submission.

10. Raymond Schroeder, the individual who did skeleton examinations was aware of the dose levels of the fetal skeleton specimens, There is no identification on the body of the vials holding the skeletal specimens; the fetus number was marked on the vial cap. This method of identifying fetal skeleton specimens in vials could cause a mix up. Photo number 1 of Exhibit 39 illustrates their method of identification of the skeletal specimen on the cap of the vials.

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Control Group

Number of Litters - 26
Number of Fetal Skeletons - 201
Number of Litters Examined by Dr. T. Collins - 5
Number of Fetuses Examined by Dr. T. Collins - 34

Fetus Numbers Examined:				
602	802	1402	2402	2702
603	803	1403	2403	2703
605	805	1405	2405	2705
606	806	1406	2406	2706
608	808	1408	2408	2708
609	809	1410	2409	2709
611	811			
	812			
	814			

Low Dosage Group

Number of Litters - 24
Number of Fetal Skeletons - 167
Number of Litters Examined by Dr. T. Collins - 5
Number of Fetuses Examined by Dr. T. Collins - 38

Fetus Numbers Examined:				
3102	3902	4602	5302	5802
3103	3903	4603	5303	5803
3105	3905	4605	5305	5805
3106	3906	4606	5306	5806
3108	3908	4608	5308	5808
3109	3911	4609	5309	5809
3111	3913	4611	5311	5811
		4612		
		4614		
		4615		

High Dosage Group

Number of Litters - 23
Number of Fetal Skeletons - 187
Number of Litters Examined by Dr. T. Collins - 5
Number of Fetuses Examined by Dr. T. Collins - 36

Fetus Numbers Examined:				
6102	7602	8002	8402	8702
6103	7603	8003	8403	8703
6105	7605	8005	8405	8705
6106	7606	8006	8406	8706
6108	7608	8008	8408	8708
6109	7609	8009	8409	
6111	7611	8011	8411	
		8012	8412	
			8416	

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Visceral Examinations - Study E-5

Approximately one-third of the fetuses were fixed in Bouin's solution for subsequent examination by the free hand sectioning technique of Wilson. Tissue slices were examined under a dissecting microscope. The report submitted to FDA indicates that all tissue slices from treated fetuses and from control fetuses with anomalies were transferred to polyethylene bags for temporary storage. These specimens were discarded prior to our inspection and therefore we were unable to make any examinations of their visceral sections. There were no initials to identify the individual who did the work on the visceral examination sheets (Exhibit 12). The visceral examination records do not list the respective fetus identification numbers for about 10% of the 329 fetal visceral specimens. These incompletely identified fetus specimens are identified on the visceral examination records with only the dam number and fetus sex. As an example, a fetus of Dam number 40 would be listed as 40X female. The visceral examination sheet indicates only "O.K." if no abnormalities are found in the respective visceral section. There is no examination sheet that specifies what abnormalities they are particularly looking for in the visceral sections. The individual doing the examinations was aware of dose levels of the visceral specimens.

According to the visceral examination records, see exhibit 12, a total of 329 visceral examinations were done on two days, namely Feb. 27, 1970 and March 5, 1970. Mr. Schroeder said that he did visceral sections on approximately 30 fetuses per day.

The raw data and the report submitted to FDA specifies the finding of only three anomalies. Hydrocephalus was observed in one low dose and in one high dose fetus. Hydronephrosis and hydroureter were observed in one control fetus. We noted that the original visceral examination records also specified the finding of blood in the pericardial cavity of a visceral section of fetus number 4601 and the marking, "O.K.". This finding of blood in the pericardial cavity was not in the FDA submission. There were no other specific findings listed on the rat visceral examination sheets. The results of the remaining respective fetal visceral examinations were listed simply as "O.K.". It may be interesting to note that there have been teratology studies conducted in the rat by FDA laboratories where the findings in the visceral sections are reported for at least 100 of the fetuses.

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COMPARISON OF THE LAPOROTOMY AND VISCERAL SHEETS

We uncovered at least 35 discrepancies when we compared listing of fetuses on the visceral and laporotomy examination record sheets (Exhibit numbers 8, 9, 10, and 12). Twenty-one of these discrepancies consist of listing a different sex for the respective fetus on the laparotomy and visceral examination sheets. The remainder of these 35 discrepancies include a listing of the alcohol fixative, (a) skeletal on the laporotomy sheets for fetuses that are listed on the visceral examination sheets or a listing of Bouin's fixative, (b) visceral on the laparotomy sheets for fetuses that are not listed on the visceral sheets. The following tabulation illustrates these discrepancies.

Visc. Exam Sheet	Lap. Exam Sheet	Comment
Not listed	4413 (B) Female	
4412 Female	4412 (A) Female	4412 is not in skeletal inventory
6104 Male	6104 (B) Female	
6110 Female	6110 (B) Male	
5801 Female	5801 (B) Male	
5810 Male	5810 (B) Female	
2110 Male	2110 (B) Female	
2113 Male	2113 (A) Male	2113 is not in skeletal inventory
1001 Female	1001 (B) Male	
1013 Male	1013 (B) Female	
7710 Female	7710 (B) Male	
7713 Male	7713 (B) Female	
Not listed	3012 (B) Female	3012 is not in skeletal inventory
3013 Male	3013 (A) Male	
2701 Male	2701 (B) Female	
2704 Female	2704 (B) Male	
804 Male	804 (B) Female	
813 Female	813 (B) Male	
2910 Male	2910 (B) Female	
Not listed	7212 (B) Female	
7217 Female	Not listed	DAM 72 had only 13 fetuses, it might refer to fetus #7212
3201 Male	3201 (B) Female	
6401 Male	6401 (B) Female	
6413 Female	6413 (B) Male	
3304 Male	3304 (B) Female	
3310 Female	3310 (B) Male	
1307 Female	1307 (B) Male	
1312 Female	1312 (A) Female	

LCT C 6% L 6% H 4%

A total of 6 fetuses are listed for Dam #13	A total of 4 fetuses are listed in Bouin's (visceral)	
Not listed	5504 (B) Male	5504 is not in skeletal inventory
5506 Female	Not Listed	Dam #55 had only 5 fetuses
A total of 6 fetuses are listed for Dam #39	A total of 5 fetuses are listed in Bouin's solution for Dam #39	
Not listed	9010 (B) Female	We were unable to locate any of the skeletal fetuses for Dam #90 during our inventory of skeletal specimens
Not listed	9012 (B) Female	
9011 Female	9011 (A) Male	

Study S-89

PT-1218675 - An evaluation of embryotoxic and teratogenic potential in the mouse - Aspartame (SC 18862) Seg. II

Before Dr. Collins, Bureau of Foods examined the visceral sections of this study it was brought to the attention of Searle's attorney, namely Mr. Roger Thies that some damage may occurr to these sections. The sections had been previously examined and it is a fact that these kinds of sections tend to come apart with age. These sections are approximately two years old.

Mr. Thies requested that official authorization in writing be given to Searle before Dr. Collins examined the visceral sections. Clearance and authorization was given by Mr. Richard Ronk, Director Division of Food and Color Additives. Dr. Collins was given authorization to examine the visceral sections of this study (S-89) in the company of a Searle teratologist. Dr. Collins agreed to inform Searle's teratologist the results of his readings, (see exhibit 41).

Date Initiated: Protocol Finalized - January 15, 1975
The first recorded body weight - Pebruary 27, 1975
The first recorded date of food consumption - Pebruary 27, 1975

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Date Completed: Last body weight - April 14, 1975

Last food consumption - April 14, 1975

Final date on visceral exam worksheets - June 18, 1975; First date - May 28, 1975

Vondruska's notation on visceral examination of fetus 41101 female - 6/24/75

Dates Recorded on Reverse of Laporotomy Sheets For Skeletal Exams: The majority of the dates are either May 19, 1975 or June 4, 1975. Six fetuses of Dam 108 are listed with a skeleton exam date of 6/3/75.

Dates Recorded on Reverse of Laporotomy Sheets for Visceral Exams: May 28, 1975 and June 4,5,6,12,15,16,17 and 18

Date on Cover Sheet of Final Report Submitted to FDA: July, 1975

Animals Used: (\ Breeding Labs
Random bred albino mice, female CD-1 strain and
Random bred albino mice, males - proven breeders

36 females - Control
36 females - Low Dose
36 females - Medium Dose
36 females - High Dose

A copy of the purchase order for these females is attached as Exhibit #21.

Mating Procedure - natural mating; detection of copulatory plug designated as day 0 of gestation

Dose Levels:	Concentration of SC-18862 in Diet (%)	Intended Daily Dose (Grams per Kilogram)
Low	.75%	- 1 GPK
Medium	1.5 %	- 2 GPK
High	3.00%	- 4 GPK

Number of Pregnant Mice:

Control: 27
Low Dose: 25
Med. Dose: 27
High Dose: 21

We noted that the protocol specifies that body weights will be made on gestation days 1, 4, 6, 13, 15, and 18. The body weights in the FDA submission were recorded on gestation days 0, 1, 3, 6, 13, 15, and 18.

Scope of the Investigation - E-89

We began a comprehensive review of Study E-89, PT 1218575 on 8/12/77, after the investigation of E-5, PT 851570 was essentially completed. This additional coverage was in accordance with authorization received from the Bureau of Foods.

We began our review by supervising the copying of all raw data stored under FDA seal at Searle Laboratories. These records include the following principal items:

1. Copy of protocol entitled Final Protocol For a Pre-clinical Safety Study of SC-18862 Path-Tox Proj. No. 1218575 (Exhibit 18).
2. Copies of laparotomy sheets - The reverse of the laparotomy sheets include visceral examinations and some of skeletal examination findings. (Exhibits 26-29)
3. Body weight data (Exhibit 24)
4. Food consumption data (Exhibit 25)
5. Visceral examination work sheet (Exhibit 31)
6. Skeletal examination data (Exhibit 30)
7. Statistical data (Exhibit 35)

The investigators audited this raw data by reconstructing the information submitted in Summary Tables. We verified total number of animals on test, independently tabulated and compared information on Summary of Uterine Implantation of all groups, verified maternal body weights, food consumption and calculated g/Kg of test substance administered. Dr. T. Collins examined selected skeletal and visceral sections.

Personnel for Study #E-89

This study was conducted by the following individuals:

1. Dr. James Vondruska - Senior Research Investigator
2. Alan L. Mitchell - Teratologist
3. Gail Kirby - Research Technician
4. Ray Schroeder - Senior Research Assistant
5. Jeanne Thompson - Research Technician

The Curriculum Vitae for Dr. James Vondruska, Alan L. Mitchell, and Gail Kirby are attached as Exhibit #16. The Curriculum Vitae for Raymond Schroeder is included with Exhibit #1.

Our review of CV's established:
Dr. James Vondruska is a licensed veterinarian and is certified by American College of Laboratory Animal Medicine. He has been employed by Searle Laboratories since March, 1973.
Dr. Vondruska said that he was responsible for submitting the final report on E-89, PT 1218S75.

Alan L. Mitchell is a graduate of Southern Illinois University and completed some graduate work at DePaul University, Chicago, Illinois. Mr. Mitchell has assisted in supervising the teratology laboratory since 1971. Regarding the conduct of E-89, Mr. Mitchell was responsible for preparing the Treatment Mixture and for supervising the maternal body weighings and food consumption.

Raymond Schroeder has a Masters degree in zoology from the University of Illinois. He worked at Searle Laboratories as a teratologist from December, 1967 until May 2, 1975. With regard to E-89, Mr. Schroeder was responsible for training Gail Kirby in teratology

and for supervising the hysterotomy examinations. A detailed account of our June 22, 1977, interview with R. Schroeder can be found in a subsequent portion of this report.

Mrs. Jeanne Thompson, Technician, had very limited duties on this study (S-89). She was responsible for taking maternal body weights and food consumption.

Gail Kirby, Research Technician, has been employed at Searle Laboratories since August, 1974. She played a major role in the conduct of S-89. In this experiment she was responsible for performing all of the visceral examinations and the skeletal exams. She received her training in teratology from Ray Schroeder.

Mrs. Kirby's educational qualifications include the following: Mrs. Kirby graduated from Elgin High School, June, 1971 and attended Loyola University for three years where she acquired 161 semester hours of credit.

During an interview with Mrs. Kirby she described her responsibilities in conducting this experiment to include the following: Mrs. Kirby told the investigators that she assisted in performing hysterotomies, weighed fetuses, sexed the fetuses, recorded gross observations, performed crown rump measurements, and recorded uterine distribution.

She stated that in S-89 she was also responsible for preparing and staining fetal skeletons and visceral sections. Mrs. Kirby initially reported that Ray Schroeder read the visceral sections on this experiment but later corrected the statement saying she examined the visceral sections.

Mrs. Kirby also told the investigators that she personally examined skeletons on this experiment and Dr. Vondruska had checked some of her observations. A detailed account of the two interviews that we held with with Gail Kirby is included in subsequent sections of this report. Curriculum vitae for J.F. Vondruska, A.L. Mitchell, and G. Kirby are attached to this report as exhibit VII. We requested the curriculum vitae for J. Thompson on numerous occasions but we were told that no formal curriculum existed for this individual.

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Methods and Facilities

Interviews with Dr. James Vondruska and Alan Mitchell on 5/24/77 established the following: Dr. Vondruska stated that Vet Service Department was responsible for animal care. The diet was prepared by Alan Mitchell and he was assisted in taking body weight and food consumption data by Joanne Thompson, Research Technician.

The investigators made an on site visit to animal facilities on 6/2/77. Dr. Vondruska identified Room 328 in J Building as the room where study E-89 was conducted.

We were shown the type of cage and feeder used. We noted that this room was equipped with temperature and lighting control. It had only one doorway for entrance and exit.

We were informed that individual female mice used in E-89 did not bear any unique identification mark after breeding. The mice were marked with tail coloring for the respective groups. Breeding cage cards and individual female cage cards are submitted as Exhibit 23. Record of daily observation would be recorded on these cards. We noted one observation on the individual cage card for animal 117 "extensive bleeding from vagina on 4/5/75". The observation is recorded in the Submission on Table No. 4 Summary Uterine Information Data Control Group.

Compound Formulations - E-89

The test substance being evaluated in this Segment II Teratology Study is L - aspartyl - L - phenylalanine methyl ester (SC18862) (aspartame) Lot 59687, Q.C. 0475. This powdered SC 18862 was administered by dietary incorporation in powdered [illegible]Rat Diet from gestation day 6 through 15. The following intended dose levels were fed the test animals.

	INTENDED DAILY DOSE LEVELS	CONCENTRATION IN RAT DIET (ACTUAL)
Low Dose	1.0 grams/Kg	.75%
Mid Dose	2.0 grams/Kg	1.50%
High Dose	4 grams/Kg	3.00%

The animals actually received approximately 40 % more than the originally intended doses.

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Searle Laboratories did not maintain batch records of the treatment/diet mixtures, nor assay for potency, homogeneity or stability. In addition we were unable to establish that the personnel kept any note books or any other written record on the method of diet preparation.

Dr. Jenkins was able to locate some uniformity of mix studies in the [redacted] mixer on a different active ingredient in Rat Chow, namely SC-10295. These results of analysis are dated March and April of 1976 and are attached as exhibit 33. Although these studies do not substantiate the uniformity of mix of SC18862 with [redacted] Rat Chow, they are submitted for informational purposes.

The protocol for E-59 specifies as the mode of administration for S.C. 18862 to be admixed W/W in the diet. Dr. William Jenkins furnished the investigators with a copy of a label for the Purina Rat Chow, stating that this was the only information available as to the composition and/or specifications on the feed (Exhibit 22). Dr. Jenkins also accompanied us to the Diet Preparation Room and identified the mixer. It was a Hobart Model C-100 T with a mixing bowl of about 3 gallons capacity. Dr. Jenkins told us that there were no assays on these mixtures of S.C. 18862 with Purina Rat Chow.

Alan Mitchell told us during his interview on 5/24/77 that he was responsible for preparing the diet mix. He described his mixing procedures as follows: The diet was made up in 1000 gram batches. Approximately 500 grams was placed in the mixer bowl and then the appropriate amount of S.C. 18862 was added. Then the remaining amount of the Rat Chow was added and the contents were mixed for 10 min.

Although they did not assay the mixture of S.C. 18862 with basal diet, they did assay the test substance S.C. 18862. We obtained analytical records for their Quality Control original assay of Aspartame Lot 59687 S.C. CC075. (Exhibit #19 and Exhibit #20)

It was noted that the analyst made a decimal point error in his original work book calculations when assaying for potency. (Exhibit 19) The error was caused by using 1500 mg. instead of 150 mg. quantity in the equation and multiplying by 100 instead of 10,000. If the calculation for potency is made in accordance with the equation listed with their analytical method

Exhibit 20 and correct placement of the decimal point, the calculation indicates satisfactory potency of aspartame (S.C. 18862) for their sample weight of .1500 gms. It was explained to the investigators by Mr. Aspinall that they weighed out exactly 150 mgs. for this assay. He said that the equation for calculation of potency was not checked by a 2nd person.

Hysterotomy Data - B-89

Hysterotomies for B-89 were performed by Ray Schroeder and Gail Kirby between the periods of 3/17/75 to 4/14/75. Mrs. Kirby told the investigators during an interview on 5/24/77 that her duties for study B-89 included:

a. performing dissections
b. weighing fetuses
c. sexing fetuses
d. entering gross observations
e. did crown-rump measurements
f. recording uterine distribution.

During this time Mrs. Kirby was supervised by Ray Schroeder. Ray Schroeder was responsible for the external examination of the fetuses (see R. Schroeder interview). We authenticated the hysterotomy data by reconstructing a chart from all of the raw data. We found that this information was accurately recorded and essentially the same as in the FDA submission. We verified total number of fetuses, number of resorptions, total dead fetuses, average crown-rump measurements, and body weights (exhibits 26 through 29).

We also checked accuracy of recording the sexes by comparing the data on the laparotomy sheets against the visceral exam sheets. We found no errors in making this comparison.

Exceptions:

Female #236 with gestation day 1 of March 22, 1975 delivered 4 fetuses 4/8/75, three viable and one non viable. This appears to be a full term for the fetuses to gestation day 18. The FDA submission states that this female delivered prematurely on gestation day 18. No pups were saved for examination due to their condition. The data regarding these dead pups was not included in their calculation of means. A similar type situation was

recorded for female #308 with gestation day 1 of March 3, 1975 who delivered 10 fetuses on March 20, 1975, one cannibalized and 9 intact fetuses. This also appears to be a full term for the fetuses to gestation day 18. No pups were saved for examination and data regarding these pups was not included in their calculations of mean values. This was probably a correct procedure because the plug of the dams was missed and hence the animals dosed on incorrect days. In Dr. Collins' opinion it would have been better if these litters had been examined and weighed and the records kept.

The uterine implantation data listed in tables in the FDA submission included: data on number of fetuses; number of resorptions; sex distribution; mean fetal body weight; mean fetal crown-rump measurements; and number of fetal examinations. We noted the following discrepancy when we compared these tables on uterine implantation in the FDA submission with their raw data. The averaged female fetal crown rump measurement of animal #307 is reported on table number 4 as 2.5; it should be 3.1.

Food Consumption

Copies of the body weights and food consumption records for study E-89 are attached as Exhibit numbers 24 and 25. Our calculation of the raw data for at least one third of the food consumption quantities listed in the FDA submission indicates agreement with a statement on page 9 of the FDA submission that the pregnant animals of the low, medium and high dose groups consumed approximately 40% more than the originally intended doses of 1.0, 2.0, and 4.0 gm per kilogram.

Alan Mitchell said a 4 oz. glass jar was used as the food container. A paper under each jar was used to collect the spillage; the feed was dumped back into the feeder jar. Our calculation of original food consumption data uncovered only the following 5 discrepancies from values listed in the FDA submission.

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Animal No.	Gestation Day	Amt. of SC 18862 consumption listed in FDA submission (grams/kg)	Amt. of SC 18862 consumption according to our calculations
405	10	4.4	4.6
232	13	1.6	2.0 * - there is an asterisk on F.C. record for day 13 - and the handwritten comment "I am not sure about spillage."
226	13	1.2	1.76
232	10	1.0	1.6 - Calculated on body wt. - day 11; day 10 body wt. was not recorded
201	6	1.3	2.25

amt ingested

Mrs. Jeanne Thompson, Research Technician, was interviewed 5/26/77. She said that she was responsible for taking body weight and food consumption data. She was supervised in these operations by Alan Mitchell. Mrs. J. Thompson said that the dosage levels of the mice were identified by marking their tails with a specific color.

Mrs. Thompson described how the animals were fed and how the weighings were done. She said that the cages were pulled out and the animals and feed container weighed on the Intec. Afterward the food was added and the container reweighed. According to her, the diet mix was stored in a labelled plastic container.

Mrs. Thompson told the investigators that where an asterisk appears on the Intec print out under food consumption, it meant that their weighing indicates spillage that is not usable for calculating food consumption. She could not recall whether or not the animals were weighed and fed at the same time each day.

Skeletal Examinations Re: Study #1-88

The results of their skeletal examinations are partly on the reverse of the laparotomy sheets. (Exhibit #26 thru #28). The research technician included a record of the date of the skeletal examination and the respective fetus number on the laparotomy sheets. However, the findings listed for the respective skeletal fetus on the back of the laparotomy sheet are for the most part incomplete because the research technician listed only the findings that she considered relatively unusual. They also have examination date in their tabular skeletal reporting format (Exhibit #30) by litter number and not by individual fetus. This tabular skeletal format is not dated. There are no initials or signatures to identify the individual who did the skeletal examinations.

We compared original examination records, the reverse of the laparotomy sheets (Exhibits #26 thru #28) and the tabular skeleton reporting format (Exhibit #30) with the report that was submitted to FDA (Exhibit #37). Dr. T. Collins made a detailed examination of skeletal specimens of 5 litters from each dose level, and authenicated the major abnormalities in other litters. Our findings include the following:

1. The original skeletal examination records essentially agree with statements in the FDA submission. The tabular skeletal reporting format (Exhibit #30) did not clearly differentiate between the total number of sternebrae centers that were absent and the total number of "small" sternebrae centers.

2. The rudimentary structures are small projections from the first lumbar vertebrae. These structures are in essence a small 14th rib. Most animals with these structures are graded twice. They are counted as having 13 pairs of ribs as well as rudimentary structures.

3. Dr. T. Collins made a detailed examination of fetal skeletal specimens from 5 litters of each dosage group. He also scanned in detail the supraoccipital bone for poor ossification in each of the skeletal fetuses of the control and high dosage group. Details regarding this examination follow in subsequent paragraphs. His examination of the supraoccipital bone revealed the following percentage differences from the FDA submission.

Supraoccipital Bone	Control Fetuses	High Dose Fetuses
Poorly Ossified		
FDA Submission	3%	6%
Examination by Dr. T. Collins	4.46%	8.47%

Dr. T. Collins also examined fetal skeletal specimens to verify their findings of major malformations: fetal skeleton #10803 with hypoplastic 4th thoracic vertebral centrum and fetal skeleton #32703, with frontal, parietal and interparietal poorly ossified, 2nd, 3rd, 4th and 5th sternebrae split, and cleft palate. He also made a rapid scan of the fetal skeleton of low dose dams #228 and #229 and medium dose dam #301 to confirm their findings.

4. Dr. Collins found some minor differences in their classification of skeletal variations. An example would be the ossification of the supraoccipital bone. A certain amount of variation normally occurs between individuals when making these types of skeletal examinations. No serious errors were found.

5. The skeletal examinations were not done blindly. The individual knew the dose levels. There is no identification on the body of each vial that each holds one skeletal specimen; the PT number 1218 and the respective fetus number are on a label on the vial cap (see exhibit 39, photo 1).

6. We made a physical inventory of the skeletal fetuses and could account for all of them with the exception of one fetus from the high level (#42210). This was reported in the FDA submission.

7. There are no dates of examination of the skeleton tables (exhibit 30). On the back of the laparotomy sheets, the major skeletal variations are listed. Most of the skeletal examinations are dated 5/19/75 and 6/4/75. It would be impossible for one individual to do a complete skeletal examination of over 500 fetuses in 2 days. It is unclear over what period of time these fetuses were read.

8. Gail Kirby, the research technician who performed the visceral and skeletal fetal examinations for study #1-89 completed about 3 years of college with job related courses that included embryology, comparative anatomy, zoology and genetics. She started employment with Searle Laboratories in August of 1974 and performed visceral and skeletal exams for study E-89 in in May and June of 1975. This was the only study where she performed the visceral exams. She stated that her-on-the job training was about 3 months. We obtained copies of two Searle Training Manuals for fetal soft tissue and skeletal examination (exhibit #32). Roger Thein, attorney cautioned us that they couldn't determine the date when these training manuals came into existence. Therefore they couldn't be considered SOP Manuals for this study. This instruction manual does not have skeletal photos referred to in the manual.

Details for Scanning of Supraoccipital Bone in Control And High Dose Group

A selective examination was made by Dr. T. Collins for poorly ossified supraoccipital bone in all of the control (157) and high dose (118) fetal skeletons. Dr. T. Collins found ten skeletal fetuses that had a poorly ossified supraoccipital in the high dosage group; 40103, 40110, 40204, 40713, 40711, 40708, 41103, 41106, 41508 and 41603. The summary of fetal skeletal examination data in the FDA submission states that they found 7 fetuses with a poorly ossified supraoccipital bone in the high dose group. Dr. T. Collins confirmed their findings in 7 of these skeletal fetuses. He also uncovered poorly ossified supraoccipital bone in three additional skeletal fetuses in the high dose group, namely 40103, 40110 and 40713. Dr. T. Collins found seven skeletal fetuses with a poorly ossified supraoccipital in the control group; 10102, 10205, 10206, 12302, 12305, 13203, and 13208. The summary of fetal skeletal examination data in the FDA submission states that Searle found 5 skeletal fetuses from the control group with a supraoccipital bone that was poorly ossified. Dr. T. Collins confirmed their findings of a poorly ossified supraoccipital bone in 4 of the control skeletal fetuses. He did not agree with their finding of a poorly ossified supraoccipital bone in fetus number 10905.

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Detailed Examination of Skeletal Fetuses By Dr. T. Collins

Control Group

Number of Litters - 25
Number of Fetal Skeletons - 157
Number of Litters Examined by Dr. T. Collins - 5
Number of Fetuses Examined by Dr. T. Collins - 30
Fetus Numbers Examined:

10102	10805	11202	12302	12402
10103	10806	11203	12303	12403
10105	10808	11205	12305	12405
10106	10809	11207		12406
10108	10811			12408
10109	10813			12409
10802				12411
10803				12412
				12414

Low Dosage Group

Number of Litters - 24
Number of Fetal Skeletons - 158
Number of Litters Examined by Dr. T. Collins - 5
Number of Fetuses Examined by Dr. T. Collins - 27
Fetus Numbers Examined:

20202	21002	21802	23402	23502
20203	21003	21803	23403	23503
20205	21005	21805	23405	23505
20206	21006	21806	23407	23506
20208	21008	21808		23508
	21010	21810		23509

Medium Dosage Group

Number of Litters - 25
Number of Fetal Skeletons - 163
Number of Litters Examined by Dr. T. Collins - 5
Number of Fetuses Examined by Dr. T. Collins - 34
Fetus Numbers Examined:

30602	30702	32702	33102	33502
30603	30703	32703	33103	33503
30605	30705	32705	33105	33505
30606	30706	32706	33106	33506
30608	30708	32708	33108	33508
30609	30709		33109	33509
	30711		33111	33511
	30712		33113	

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High Dosage Group
Number of Litters - 20
Number of Fetal Skeletons - 118
Number of Litters Examined by Dr. T. Collins - 5
Number of Fetuses Examined by Dr. T. Collins - 23
Fetus Numbers Examined: 40201 41102 41201 41402 43002
40204 41103 41204 41403 43003
40207 41105 41207 41405 43005
41106 41406 43006
41408 43008
41409
41411
41412

Visceral Examination-E-89

Approximately one-third of the fetuses from each litter were fixed in Bouin's Solution for subsequent examination by the Free-Hand Sectioning Technique of Wilson. The tissue slices were examined under a dissecting microscope. All tissue slices from control and treated fetuses were then transferred to glass vials that were filled with 70% ethanol for storage. The vials are identified with the project No. PT #1218 and the respective fetus number. These tissue slices are also identified inside each of the vials with the respective fetus number.

Our physical inventory of their visceral specimens reveals that they are in conformance with the listing of the fetuses recorded on the reverse of the laporotomy sheet (Exhibit #26 thru 29). We noted that the alcohol was evaporated in the following vials and those visceral specimens might have been damaged or destroyed: 22310, 22503, 23507, 12101, 11309, 11304, 10101, 10904, 10707, 20101, 20800, 12304, 12301, 12807, 20207, 20107.

Gail Kirby who did both the visceral and skeletal examinations was aware of the dose level of the specimens that were being evaluated. There are no examination sheets that specify the abnormalities that are included in their examination of visceral sections.

During our interview with Gail Kirby she stated that a training manual had been provided her by Searle Laboratories. We subsequently received copies of training manuals from Roger Theis, Searle attorney. The firm was reluctant to provide these training manuals because they could not establish the date their manuals were initially used. Copies of their manuals for visceral and skeletal examinations were ultimately provided and are submitted Exhibit #32. In reviewing

these manuals we noted that they pertain primarily to rabbit and rat visceral exams and not to mouse visceral exams. Also, Searle Attorney, Roger Theis did not furnish copies of skeletal pictures referred to in the manual.

1. We noted only one discrepancy during our inventory. The soft tissue specimen from fetus #42209 female was found in inventory but the visceral exam records do not indicate that it had been examined. The laboratory sheet for L-59 indicates that the skeletal specimen of fetus #42210 was lost. There is a soft tissue exam listed for #42210 female with results of "O.K." for this fetus that was not in their soft tissue inventory.

   We compared the listing of the fetuses on the visceral (Exhibit #31) and laparotomy sheets (Exhibit #26-29) and noted that Searle correctly listed the same sex for the respective fetus on the visceral and laparotomy sheets. They also correctly specified the use of Bouin's fixative for the visceral specimens. We noted that the results of the visceral examinations for 5 fetuses of dam 124 and 5 fetuses of dam 226 are reported on the back of the laparotomy sheets (Exhibit #26 & 27), but these fetuses are not listed on the visceral exam sheets (Exhibit #31).

   Study L-59 was the only study where Gail Kirby performed the visceral exams.

   The visceral examination instruction manuals are not specific with regard to number of sections or thickness thru the heart. We were unable to ask Gail Kirby to examine these manuals to determine if she used them for training or reference. Mrs. Kirby was in her ninth month of pregnancy and was on maternity leave when we conducted our second and final interview by a telephone conference call to her home. Details regarding both the interviews are found in a subsequent section of this report.

Examination of Visceral Specimens by Dr. T. Collins

Dr. Collins examined a total of 31 visceral specimens. Photo #2 of Exhibit #39 illustrates some of the visceral examinations made by Dr. T. Collins.

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Skokie, Illinois 60076

Visceral Fetal #	Comment
32012	Dr. Collins verfied the finding of a cleft palate that was indicated in their raw data but not in their FDA submission.
40310	No Abnormalities
42207	No Abnormalities
42209	No Abnormalities
40109	Dr. Collins did not locate the section that was made for the renal pelvic area.
40301	No Abnormalities
41205	No Abnormalities
43007	They did not get enough sections.
43613	No Abnormalities
42401	No Abnormalities
42407	No Abnormalities
41906	No Abnormalities
42607	No Abnormalities
42610	Specimen was in poor condition for examination
42007	No Abnormalities
42009	No Abnormalities
40202	No Abnormalities
40206	No Abnormalities
40707	No Abnormalities
40712	No Abnormalities; but exceptionally thick sections.
41708	No Abnormalities but section of thorax was too thick, approximately 5 mm (exhibit 39, photo 2). The FDA submission stated that the slices of the thorax would be somewhat thinner than 1 mm.
20407	Dr. Collins verified the findings of a segmented uterus that was indicated in their raw data but not in their FDA submisaion. Dr. Collins also noted that there is a slight hydrocephalus of the ventricle and enlargement that is not in their raw data (exhibit 39, photo 3).
41101	Their raw data indicates that fetus 41101 has "a renal pelvic cavitation of the kidney not enlarged" and is an artifact and not a malformation. Examination of this fetus by Dr. Collins indicates an enlarged kidney with hydronephrosis (exhibit 39, photo 4)
43201	No Abnormalities
43204	No Abnormalities but in the opinion of Dr. Collins there were not enough sections thru the heart.

43207	No abnormalities but in the opinion of Dr. Collins there were not enough sections thru the heart.
41702	The specimen was broken up and was a problem to examine
41703	The specimen was broken up and was a problem to examine
41705	No Abnormalities
41706	No Abnormalities
41709	No Abnormalities

Dr. Collins estimated that approximately 50% of the fetuses had one or more visceral sections that were too thick (exhibit 39, photo 2). It may be significant that their findings in their total of 367 visceral sections pertained to only three fetuses (Exhibit #31). Dr. Collins noted in some cases that they missed the renal pelvic area. There is a possibility that some of the sections might have disintegrated or some of the sections might not have been placed in the vial at the time when they were originally examined by Searle Laboratoris.

Statistical Evaluation

Attached as exhibit 35 is a memo from Mr. Dennis I. Ruggles, Department of Mathematics, HFF-110 to Dr. Collins HFF-155 regarding an evaluation of the statistical methodology employed in this study (E-89). An actual statistical review was not performed. In Dr. Collins' opinion this statistical review of the FDA submission showed that the methodology employed in this study were essentially correct. The comments made by Mr. Ruggles concerning this methodology were minor (please refer to exhibit 35).

Interviews with Gail Kirby

An initial interview was held with Gail Kirby, research technician, on 5/25/77. Mrs. Kirby was reinterviewed on 6/7/77 by telephone in order to obtain additional information. We felt this was necessary because Mrs. Kirby played a major role in the conduct of E-89.

The second interview was held by a conference phone from Searle Laboratories to Mrs. Kirby at her residence.

The interviews will be reported in question and answer format to point out differences between the two interviews. Portions of this information have been reported under the respective heading. On 6/2/77 Richard Viktora, attorney told us that Gail Kirby had reconsidered her first interview and had now decided that on study E-89 she had performed the visceral examinations.

Interview with Gail Kirby 5/25/77

Q. What was your job in E-89?

A. I worked as a Research Technician in Teratology. My duties included performing hysterotomies, preparing fetuses in Bouins, preparing skeletons for staining, cutting visceral sections and recording data.

Q. Describe your hysterotomy duties.

A. These included:
1. Making dissections
2. Weighing the fetuses
3. Sexing the animal
4. Noting the gross abnormalities
5. Crown rump measurements
6. Uterine distribution of fetuses

She did the entire hysterotomy, she generally wrote her findings on the laparotomy sheet but occasionally she might have received help with the transcription.

Q. How were the Wilson sections prepared?

A. I cut the sections for someone else to look at. The sections were made as follows:

1. Six sections through head
2. 5 or 6 through thorax
3. 2 through the kidney

Q. Who evaluated the visceral sections?

A. Ray Schroeder evaluated viscerals. I may have transcribed. (Note: she subsequently stated that she made a mistake in this initial interview and that actually she did those visceral examinations).

Q. How long would it take Ray Schroeder to evaluate visceral specimens represented in 2 visceral exam sheets dated 6/17/77?

A. It would take all day. (25 litters)

Q. How did you prepare fetuses for Wilson sections?

A. Put the fetuses in bouin's for 2 or three weeks, then rinse in tap water 2 or 3 times and then cut the fetus 2 at a time on a plate. (She made a record of the fetus number).

Q. How were the fetuses sexed?

A. The sexing on the visceral was done by identifying the organ.

Q. Did you use a checklist when performing visceral exams?

A. No we did not use a form.

Q. Describe your procedure in doing visceral exam.

A. I took the fetus out of the jar which contained water. Then I sat down where I had paper on my right side. Ray Schroeder would then evaluate the visceral sections.

Q. Why don't the work sheets show more Bouins Stain?

A. I used gloves.

Q. Who did the skeletons on E-89?

A. I have done skeletons examinations, but I don't remember if I did these.

Q. Showed her the skeletal results.

A. "I did the skeletons on E-89".

Q. On your skeletal closures, what do you consider normal?

A. This criteria is given in our manuals.

4 - 75%-100% ossified
3 - 50%-75% ossified

Q. How did you assess the skull closure? Did you do a real screening job?

A. The closure was what was mostly done.

Q. Could you say you screened the frontal bone or parietal bone?

A. "I hope I did".

Q. How many autopsies could one person do in one day?

A. 30 autopsies per day. I started at 8:00 am.
(Gail said that she did not kill the animals at one time, she did the killing over an extended period of time).

Q. Who else assisted in the skeletal exam?

A. I was the only one who did skeletal exam.

Q. In doing this skeletal exam, is it fair to say that you knew what level you were looking at?

A. Yes, we knew the levels.

Q. Can you describe a 5th sternum.

A. It is always smaller, it is the size of a pin head.

Second Interview with Gail Kirby, 6/7/77
(Telephone interview)

Q. Give us your educational background.

A. I attended Loyola University until June, 1974 and accumulated some 100 hours credit at Loyola. My biology courses included comparative anatomy, embryology, microbiology, 2 inorganic chemistry courses, one organic chemistry, 2 physics, plus usual liberal arts.

Q. Did you receive a college degree?

A. No, I have not received a degree.

Q. Please tell us about your work history.

A. I started in teratology at Searle in August, 1974. My supervisor was Ray Schroeder. He taught me the basics. Ray gave me material to read and did historical control animals to show me absorption and how to make skeleton specimens. We did the visceral sections according to Wilson's book.

Q. When did you start doing skeletons.

A. Probably about 3 months after I came to Searle.

Q. Tell us how you recorded skeletal data, and the reason for having the data in two places, i.e. on back of laparotomy and in skeletal summary report by dam, e.g. 41507 5/10/75. We noted that on back of the laparotomy sheets, there is a skeletal reading by fetus but it does not contain all of the data.

A. Each fetus was looked at individually and reported by dam number on the skeletal sheet. Once it was all tallied, anything that was unusual or outstanding was put on the back of the laparotomy sheet by fetus. The transcription was not done at time of original examination. I did not go back to the fetus to record the significant findings.

Q. How did you remember the observation?

A. I think that on that study, or the next we used a dictabelt. The fetuses were not examined twice. I transcribed and ultimately recorded the data on back of the laparotomy sheet.

Q. Regarding the visceral exams, what did you do?

A. The visceral data was also recorded in two places, i.e. on ruled sheets of paper and later put on back of laparotomy sheet. It was felt this made the data look better.

Q. On the skeletons, did you screen for supraoccipital bones. Also, what skull bones did you check for?

A. I think I have already answered that question for you. The bones of the skull are parietal, frontal, hyoid, upper jaw and lower jaw, nasal, mandible, maxilla, and the bones around the eyes. There is a listing of these on the tally sheet.

Q. Regarding your work experience, how many studies have you worked on?

A. 7 or 8 plus historical. I did visceral only on PT 1219 (L-69).

Q. How many aspartame studies have you worked on?

A. PT 1201, PT 1210.

Note: At this point, Roger Theis, attorney, strongly objected to the line of questioning stating that this was not relevant since Dr. Collins had not found serious objections to the skeletal exam findings.

Dr. Collins stated that it was relevant because there are very few institutions where teratology training is available, consequently in house training has to be provided.

Dr. Collins asked whether or not Searle had provided a training manual giving instruction for visceral and skeletal examination. Gail Kirby told us that a manual had been available and that it contained pictures of visceral sections.

Mrs. Kirby stated that she did some controls, during which time Ray Schroeder would point out unusual findings. She stated "Ray Schroeder taught me what was a normal condition and what was not. He also taught me what to look for when making these examinations."

This concluded the telephoned interview.

Interview With Raymond Schroeder

This interview was conducted at the | on June 22, 1977. | in

Q.: What was your role in study E-5? Who else was involved?

A.: I was supervisor of the group which included 2 technicians, Donna Helms and Margaret Faber Hoppenrath. I did not kill the animals but did examine the animals for external abnormalities. I read skeletons and read visceral sections after they had been cut. Donna Helms killed the animals, recorded the observations, and the food consumption, and made up the diet. Donna Helms made the crown-rump measurements by stretching the fetus out on a piece of paper towel, making two marks, and reading the distance with a | caliper. Margaret Faber Hoppenrath also killed animals, made up the diet, did crown-rump measurements, and measured food consumption.

Q.: Did you do the caesareans at the same time each day?

A.: Yes, around 10 in the morning.

Q.: What was the approximate age of the males?

A.: I have no idea of their age. The males were proven males from an in-house colony which had been used in previous studies.

Q.: Did you mix the diet?

A.: Yes, I did it initially. The aspartame was sieved because it had a tendency to ball up. The chow was not sieved. The manner of mixing was: a little chow was put into the bowl, aspartame was added and mixed for approximately 2 minutes, then the rest of the chow was added and mixed. The meal had larger particles than the aspartame and the meal was not ground. After the diet was mixed, there was no balling of the aspartame.

Q.: Were any batch records or reserve samples kept?

A.: None

Q.: How much meal was mixed up at one time?

A.: I don't know how much was mixed up at one time.

Q.: Describe the type of mixer and its location.

A.: It was a Hobart mixer, approximately 2 feet high, of approximat 10 gallon capacity. It was located on the third floor in the diet mixing room.

Q.: Was there any difference in the particle size of the aspartame and the chow?

A.: The finished mixture was homogeneous in appearance but lighter in color than regular chow. There was no balling and no riping. In my opinion the rats could not discriminate between chow and aspartame.

Q.: How were the animals placed on the racks?

A.: The animals were put on racks as they got pregnant. The racks were horizontal and the animals were put on in random fashion.

Q.: How were the animals identified in study F-9?

A.: The females were ear-punched. (He did not remember how the males were marked).