MEMORANDUM

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

TO : Howard R. Roberts, Ph.D.
Acting Director, Bureau of Foods (HFF-1)

DATE: September 28, 1977

9/28/77

FROM : Bureau of Foods Task Force

SUBJECT: Authentication Review of Data in Reports Submitted to the Food and Drug Administration Concerning Aspartame.

BACKGROUND

A petition proposing the use of aspartame as a food additive was received from G.D. Searle and Company on February 12, 1973. The petition was regulated on July 24, 1974, to provide for the limited use of aspartame in foods and beverages. However, preliminary results of an Agency audit of the records of some of the animal studies presented by the petitioner raised doubts about the authenticity of the data which had been used to establish the safety of the additive. The questions were serious enough that an order announcing a stay of effectiveness of the aspartame regulation was published in the Federal Register of December 5, 1975. Subsequently, efforts by the Agency to act as a "third-party" participant in a contract between Searle and an outside group to authenticate the studies were not successful. This contract would have involved the examination for authenticity of 15 "pivotal" (i.e. integral to the approval decision) and related studies on aspartame.

In lieu of the contract approach a decision was made to implement a direct inspection of certain non-clinical studies submitted to FDA in support of food additive petition No. 3A2885. This investigation began on April 25, 1977, and encompassed the authentication of all raw data and summary data relating to studies jointly chosen for review by the Bureau of Foods and EDRO. The studies selected for this authentication review are:

1. E-5 (P.T. #851S70), Evaluation of Embryotoxic and Teratogenic Potential in the Rat, conducted with SC-18862 (aspartame).

2. E-89 (P.T. #1218S75), Evaluation of the Embryotoxic and Teratogenic Potential in the Mouse, conducted with SC-18862 (aspartame).

These 2 studies were discussed in the EIR submitted on 7/18/77.

3. E-77/78 (P.T. #988S73), 115 Week Oral Tumorigencity Study in the Rat, conducted with SC-19192 (diketopiperazine - DKP).

This study was discussed in the EIR submitted 8/7/77.

The investigating team was composed of experienced field investigators supported as required by Bureau of Foods Scientists. Details of the investigation and the results obtained are provided in the Establishment Inspection Reports (EIRs) submitted on 7/13/77 and 8/7/77. A Bureau of Foods Task Force was constituted to review the EIRs and related materials in order to conclude whether the data submitted to FDA by the petitioner could be considered authentic.

BUREAU OF FOODS' REVIEW

OBJECTIVES

The Bureau's Task Force reviewed the aforementioned three studies with the following objectives:

1. To compare the findings in the EIRs with the raw data contained in the exhibits and final reports.

2. To determine whether the raw data, summary reports, and all related materials are accurately reflected in the final reports which were submitted to the FDA.

3. To determine whether the differences between data submitted to FDA and the original raw data, as noted in the EIRs, are serious enough to invalidate the studies.

SUMMARY

Several apparent deficiencies or "issues" were brought out in the two EIRs. These needed resolution before conclusions about the integrity of the three studies investigated could be determined. One finding in the examination of the chronic study (E-77/78) was the possible nonhomogeneous nature of the test substance. This issue is discussed in Appendix A, Issue 10. In addition, the final report of this study (E-77/78) submitted by the petitioner does not include some of the histological findings of neoplasms which were documented in the raw data. This issue is discussed in Appendix A, Issue 7.

In the teratology studies E-5 and E-89, the examination and reporting of visceral findings were considered to be somewhat inadequate.

In the case of E-5, there were no visceral specimens available for re-examination by the FDA teratologist. Specimens did exist, however, for study E-89, but 50 percent of these were so thick (5 mm, while the protocol specified 1 mm thick) as to preclude observation of abnormalities. The evaluation of existing specimens of E-89 by a FDA teratologist did not differ significantly from the results in the submission to FDA. Discussion of these and other issues concerning studies E-5 and E-89 are in Appendix B.

The above-noted and all other discrepancies highlighted in the subject EIRs concerning studies E-77/78, E-5 and E-89, and the significance of these differences are addressed in the attached Appendices A and B.

CONCLUSIONS

The conclusions of the Bureau scientists follow:

1. The differences noted by the investigators in the EIRs between the data submitted in support of the food additive petition and the raw data were generally accurate.

2. The differences observed and documented between the raw data and the data submitted to the Agency are not of such magnitude that they would significantly alter the conclusions of the studies.

3. Due to the lack of raw data (visceral specimens) in the E-5 study, it cannot be determined whether all of the raw data are accurately reflected in the submission to FDA. However, the data that are available were accurately reflected in the final report.

4. In the E-77/78 study, the question of whether the diet was homogeneous cannot be conclusively resolved. Although there is no doubt that the animals ingested the DKP, it cannot be determined with certainty whether the intended doses were, in fact, ingested.

5. The investigation revealed a number of practices which were considered as significant deviations from acceptable procedures for conducting non-clinical laboratory studies.

6. Based on the Bureau of Foods' evaluation of the differences between the original and submitted data, as discussed in the EIRs, the three studies appear to be authentic.

Linda L. Taylor, Ph.D. HFF-335

Vivian Goldberg, Ph.D. HFF-335

M. Jacqueline Verrett, Ph.D. HFF-156

Patrick M. Siu, Ph.D. HFF-185

Neil Sass, Ph.D. HFF-185

APPENDIX-A. E-77/78 (P.T. #988S73), 115 Week Oral Tumorigenicity Study in The Rat - Diketopiperazine.

ISSUE 1:

A comparison of individual organ weights (Appendix 1, Table 5 in the submission to FDA (Vol. 1, pgs 222-226)) with the original data on the gross pathology sheets revealed eleven (11) errors in transcribing the raw data from the pathology sheets to the tables in the submission to FDA. Calculations using values from the pathology sheets indicated that the transcribed numbers were used in calculating the average weights of the organs listed in Tables 9-9A of the submission.

COMMENT 1:

Calculations were performed using the original data with the following changes observed:

Table I

Organ	Animal No.	Wt. shown in submission	Wt. recorded in raw data	Group mean value Reported	Corrected
1-Kidneys	A12CM	3.75g	3.45g	4.96g	4.95g
2-Ven. Prostrate	L28LM	747mg	474.7mg	642mg	625mg
3-Kidneys	E14HM C02HM	11.74g 1.46g	4.746g 4.259g	4.25g	4.01g*
4-Uterus	B20HF	1115mg	1155mg	882mg	884mg
5-Ovaries	F17CF	36.7mg	233.5mg+ & 36.7mg	133.6mg	142.98mg
6-Kidneys	C01MM	9.40g	9.219g	4.63g	4.62g

*The corrected value (4.01g) is significantly different ($p < 0.025$) from control value (4.25g).

+The 2 values in the pathology sheet should have been added; instead, only one value (ovary) was submitted.

The four other differences between the values submitted and the raw data were not deemed of sufficient magnitude to warrant recalculation. It was determined that only the value for the high dose kidney weights was altered significantly from what was reported in the submission. The corrected kidney weight (4.01 grams) was found to differ significantly ($p < 0.025$) from the control kidney weight. The reported value for this high group was also lower than controls, but was not statistically significant. This difference does not appear to significantly alter the submitted data.

ISSUE 2:

A comparison of the hematology and urinalysis data revealed 21 differences between the submitted values and those in the original data (EIR, Table 4, p.54).

COMMENT 2:

Recalculation using the raw data values resulted in the following changes:

Table II

Animal No. (day)	Parameter*	Submission value	Raw data value	Group mean value Reported	Corrected
B15LF (day 364)	BUN	30.0	3.0	11.8mg/DL	7.3mg/DL
E17MM (day 734)	RBC	10.12	7.32	8.42x10^6/CMM	8.28x10^{6}CMM
E10MM (day 734)	RBC	8.20	10.12		
E15LM (day 734)	RBC	34.00	7.73	11.85x10^6/CMM	7.55x10^{6}CMM
B05MF (day 42)	LYM	90	80	84.8%	83.2%

*BUN-Blood Urea Nitrogen
RBC-Red Blood Cells
LYM-Lymphocytes

The corrected values were not found to differ statistically from the results reported to FDA by Searle.

Additionally, the values for PKU (urinary phenylketones) were not given units in the final report, but were listed as either "0" or "1". The raw data listed values as "0", "negative", "less than 15 mg%" or "15 mg%". However, it could not be determined what these numbers referred to in the final report, since "15mg%" was sometimes listed as "1",

other times listed as "0", while "less than 15mg%" was also listed as "0" and "1".

ISSUE 3 :

A third outbreak of an unidentified infectious disease was not reported in the data submitted to the FDA.

COMMENT 3 :

The third outbreak of infection should have been reported in the submission. This third occurrence of an infectious disease (May, 1973), involved only four animals (G7CM, A3HM, F25HF, and J25MM). Records show that no increase in the death rate of any of the groups occurred during this outbreak of infection. The submitted report did include mention of two instances of infection which reportedly affected both control and treated animals with equal frequency and severity. All surviving rats received penicillin treatment, with 21 rats receiving additional treatment. Although it is unclear whether the sickness and the subsequent treatment with penicillin either mitigated or potentiated the effects of diketopiperazine (DKP), the omission of this third incidence by itself would not appear to affect the original interpretation of this study.

ISSUE 4 :

The values of the serum cholesterol levels on days 546 and 798 were not included in the submission data, although the measurements were performed and the results appear in the raw data.

COMMENT 4 :

The unreported values were calculated and were found not to differ significantly from the values reported for the other days. The submission data indicated a significant decrease in serum cholesterol that was more perceptible towards the end of the study. The evaluation of the submission by the Division of Toxicology noted this decrease in serum cholesterol level. Day 546 values were not included in the submission as called for in the protocol, but the raw data revealed that only a few

females were used because of the insufficient quantity of blood obtained from other rats. A calculation with the few values available shows similar observations as in the submitted data. The values for day 798 show the same relative decrease and, therefore, reporting the additional time periods would have strengthened this noted trend. Day 798 was not specifically required by the protocol since the original study was to last for 104 weeks. The memo extending the duration of the study to 115 weeks makes no mention of further data points to be obtained. However, a subsequent memo stated that terminal bleedings were to be done at 114 weeks (798 days).

SERUM CHOLESTEROL DETERMINATIONS

	Treatment Day						
Group#	42	92	189	364	546+	734	798+
Males							
Control	92	83	103	79		136	142
Low	86	76	91	71		126	195
Medium	92	74*	89	72		139	188
High	89	70*	75*	56*		98*	102*
Females							
Control	85	95	107	90	136 (N=2)	142	232
Low	86	80*	101	89	113 (N=2)	108*	193
Medium	76	84	88*	69	117 (N=2)	104*	153*
High	74*	86	86*	57*	87 (N=2)	58*	135*

*Significantly different from controls ($p < 0.05$)-Submission

+Values not reported in submission

*FDA determination - significantly different from controls (p 0.05)

#N=6, Unless otherwise specified

Because of the small number of animals available for day 546, these data points do not provide statistically meaningful results. The omission of the values for day 798 does not alter the results of the study.

Statistical analysis of the blood and clinical chemistry data by the Bureau's Division of Mathematics demonstrated several instances where values were reported as statistically different, while FDA's analysis showed this not to be the case, and vice versa. Since the individual values for each animal were reported to FDA, the differences in the significance of the values would not appear to alter the results of this study.

ISSUE 5 :

BUN determinations were performed at days 546 and 735, are included in the raw data, but were not reported in the submission to FDA.

COMMENT 5 :

The raw data were calculated for days 546 and 735 (Table). The determinations for day 735 were performed using only 8 females (3C, 2L, 2M, 1H). Other determinations were not made by the petitioner due to some unspecified "interference" which was noted in the raw data.

BUN DETERMINATIONS

Group[•]	Treatment Day 42	92	189	364	546[+]	735[+]
			mean, (mg/DL)			
Control						
Males	23.9[•]	19.6	9.0	2.4	5.7	
Females	18.1	18.8	17.5	10.4	11.7	21.0 (N=3)
Low						
Males	21.1[•]	18.2	18.0[•]	3.1	9.9	
Females	17.7	17.3	16.1	11.8	7.3	4.5 (N=2)
Medium						
Males	22.0	20.1	18.2[•]	5.0[•]	10.3	
Females	21.6	17.9	17.4	9.7	8.3	11.8 (N=2)
High						
Males	23.3	20.8	15.9[•]	6.0[•]	8.4	
Females	18.9	18.4	17.0	11.3	9.6	2.3 (N=1)

*Statistically different from controls ($p < 0.05$)

+Data not reported

• Each determination is the mean of 6 rats unless otherwise specified.

Although these values were not included in the submission, the omission of this data would not appear to affect the results, since the findings are similar to those for the reported days.

ISSUE 6 :

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 therefore not available for sectioning). However, in three instances (A4CM, K23CF, J3CM) a diagnosis appears in the submission to FDA.

A4CM-The post mortem evaluation sheet stated "bladder-hard yellow mass in lumen, pancreas not evaluated". Autopsy sheet states that no pancreas or bladder was submitted. The submitted report for the urinary bladder for this animal listed the microscopic diagnosis as "transitional cell carcinoma (malignant)".

K23CF-No records concerning this animal were found. However, the submitted data reported an adenocarcinoma of the mammary gland (malignant).

J3CM-The post mortem evaluation sheet stated that the testis was markedly enlarged unilaterally and cavernous hemagioma unilaterally. Autopsy sheets stated that no testis were found in the bottle. The submitted report diagnosed the testis as hemagioma (benign) and the lymphoid tissues as lymphosarcoma (malignant).

COMMENT 6 :

There is no way of knowing the origins of these tissues. However, a comparison of the incidence of these types of tumors observed in all of the groups shows that their inclusion in any of the groups would not alter the conclusions of this study. The raw data lists only one transitional cell carcinoma of the bladder as being observed in this study, and it was reported for control animal A4CM. Adeno-carcinoma of the mammary gland was observed in 7 control, 3 low, 0 medium, and 4 high dose animals. Hemagioma of the testis was observed in only one control animal (J3CM), as was lymphosarcoma.

ISSUE 7 :

Records of approximately 30 animals showed differences between the gross observations on the pathology sheets and the individual pathology summaries submitted to FDA. In several instances, observations were omitted in the submitted data.

COMMENT 7 :

In general, the inspection team pathologist's review of 20% of the slides (1/2 controls females, all of the high dose females, the 73 additional females which had masses, four additional high dose animals, and one control animal) showed agreement between his findings and those of Searle. One inconsistency included a mammary tumor found in rat F27CF which was described as a papillary cystadenoma on the individual pathology sheet and as an adenocarcinoma in the submission to FDA Some of the lesions which were not reported in the submission data could have been considered insignificant by some pathologists, although the noted omissions should have been reported. The ovarian neoplasms (animal H10CF, H19CF, and H7HF) and chronic cystitis and diffuse hyperplasia (animal D29CF) he observed, but which were not included in the submission data, were generally observed in the control group. Additionally, the omission of a mass present in animal M1LF and another uterine polyp in K9MF does not appear to significantly alter the data. It was noted in the final report to FDA (p. 90) that the tissue for M1LF could not be located; therefore, the nature of the mass could not be determined. The additional uterine polyp at the medium dose level increases the incidence from 12 to 15 percent. The dose-related incidence and the significant increase in uterine polyps at the medium and high dose levels of DKP were noted when this study was reviewed by the Bureau of Foods in 1975 (HFF-152 memo dated 04/16/75 in FAP 3A-2885).

An additional mass, present in a high-dose female (F6HF), was also not reported in the submission to FDA. The pathology sheet describes this mass (located in the left inguinal region), and records indicate that it was submitted for histological examination. However,

this animal was excluded from the study due to marked autolysis. This appears to be the only instance where an animal with a mass was excluded due to autolysis. This particular discrepancy was noted in the Commissioner's testimony before the Subcommittee on Health, Committee on Labor and Public Welfare and the Subcommittee on Administrative Practice and Procedure, Committee on the Judiciary, United States Senate, January 20, 1976. It cannot be determined why this particular animal was excluded while others in a similar condition were included. The Task Force could find no evidence that this was a deliberate attempt to influence the results of the study.

ISSUE 8:

The protocol specified that 24 organs were to be embedded for each control and high dose animal and 19 organs for the low and mid dose animals. However, in many cases the actual number of tissues embedded was less than specified.

COMMENT 8:

	# of Tissues Embedded		# of Rats not in Accord w/Protocol			Percentage
	Range	Average				
Controls	10-24	20	129	of	144	90
Low dose	12-23	19	19	of	72	26
Mild dose	4-24	18	28	of	72	39
High dose	9-25	22	51	of	72	71

A review of the raw data indicated that many of the tissues appear to have been omitted due to loss from autolysis. This loss was distributed among all groups and would not appear to be selective with regard to tissue and group. 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. The percent of each group lost to autolysis was as follows:

Group	Percent
Control	12%
Low	5%
Mid	11%
High	15%

It cannot be determined whether the results would have been altered if these tissues had been obtained before autolysis.

ISSUE 9 :

Each animal housing rack (30 animals) contained a random distribution of control and treated animals. The specific problems of feeding animals housed in the above manner (animals were not uniquely identified; only the cages were identified) were discussed in the report generated by the Task Force investigation of Aspartame in 1975/1976. The chances of administering the wrong diet to the animals are greatly increased by the use of unlabeled feeding jars arranged in rows corresponding to dose group on a cart (Exhibit 7).

COMMENT 9 :

There is no evidence available to suggest that any feeding errors occurred. However, this procedure for dosing would require adequate measures to preclude possible mix-ups. It is not possible to determine whether any dietary mix-up occurred in this study because no feeding procedures exist. However, the noted dose-related increase in uterine polyp incidence, and the decreased serum cholesterol levels suggest that diet-mix-ups may not have occurred.

ISSUE 10 :

There is evidence that the diets may not have been homogeneous. The analytical records indicate that the firm's employees may have been aware of the possibility of a nonhomogeneous diet mixture. There was a photograph of a diet mixture which showed discrete light-colored particles of varying sizes and shapes distributed nonuniformly throughout the diet mixture. In the photograph, the rat chow itself was of a rather fine granular form. A statement in the assay report of these diet mixtures of 2-16-77 indicated that these samples were not homogeneous, and that they had to be reground before they could be sampled. There is no evidence that the diets fed the rats in this study were reground. Further, it could not be determined whether these samples were

representative of the diets fed to the rats, since the batches were made up specifically for this analysis and were made in smaller amounts.

There is evidence that the diets may have been homogeneous: (1) a dose-related increase in the incidence in uterine polyps, and (2) a decrease in serum cholesterol levels with increasing dose. Additionally, there was no documentation of diet preparation or records of the amount of diet used.

COMMENT 10 :

From the available information, it cannot be determined whether (1) the diet was homogeneous, or (2) the rats ingested the intended dose levels as stated in the study. Although there is little doubt that the rats ingested some of the DEP, the levels actually ingested cannot be determined with certainty.

ISSUE 11 :

There were discrepancies in the reporting of some individual animal data:

1) It was noted in the EIR that records indicated that animal E2CM was substituted for A11CM at the scheduled 104-week bleeding, when in fact A11CM was alive at this time and should have been bled.

2) Raw data 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.

3) There were ophthalmoscopic examination records for H26MF and J29CM, but these were not reported in the submission to FDA. Both G16CM and G12CM had identical ophthalmoscopic findings on the pathology sheets, but only one examination record was found (G12CM). Only animal G16CM was reported in the submission.

COMMENT 11 :

1 - Table 2, page 164 of the submission to FDA lists E2CM in the control group for A11CM. However, the raw data sheets (bleeding record data) indicate that A11CM was bled at the scheduled time, but there is no indication that E2CM was bled. Exhibit 65 lists A11CM as dead on 11/25/73. This date is 2 weeks after the 104-week bleeding period, so it would appear that A11CM may have been bled as scheduled. Animal E2CM was probably substituted for A11CM at some later date.

2 - Exhibit 66 records A23LM as dead at 628 days (89.7 weeks). Exhibits 75a Feeder-weight data indicated that no weights were recorded from 90 weeks to the end of the study. No reason could be found in the Searle data to explain observations recorded for this animal at week 108. It would appear that a recording error occurred on this day.

3 - The raw data sheets reported bilateral superficial corneal haziness for J29CM, and anterior subcapsular opacity extending posteriorly for H26MF.

There were no unusual findings observed in any of these animals, and the differences between what is recorded in the raw data and what was submitted do not appear to alter the interpretation of this study.

ISSUE 12 :

A tissue mass was excised from a high dose animal (B3HF) during the course of the study, and this animal was permitted to continue on the study. Additionally, a skin incision was performed over masses on two low dose animals (C22LM and G25LM). These practices were mentioned in the Commissioner's testimony at the Senate hearing on the Searle investigation.

COMMENT 12 :

These procedures were performed on treated animals only. Although the tissue mass of B3HF was reported to FDA, such early excision can prevent the progression to malignancy. Further, the practice of excision was not mentioned in the submission

to FDA. The raw data indicated that the tissue masses observed in C22LM and C25LM regressed during the course of the study. Additionally, these two masses appeared approximately one week after a rodenticide had been used in the housing area. Animal B3HF and C22LM were reported in the final submission to FDA in a table of individual animals bearing histologically-proven tumors.

ISSUE 13 :

Discrepancies were observed between the clinical laboratory methods described in the submission and those actually used during the study. In some instances a procedure was changed during the course of the study.

COMMENT 13 :

Documentation of the methods actually used should have been made in the submission to FDA. However, the lack of such documentation would not appear to have jeopardized the outcome of this study. The changing of a procedure of an analysis during the course of a study is not unusual although such a change could conceivably result in differences in the apparent absolute values obtained for the concentration of the substance measured. Because comparisons are made between dose groups for a given day and not necessarily between days, this aspect would not appear to invalidate the study.

APPENDIX B. E-5 (P.T. #851S70), Evaluation of the Embryotoxic and Teratogenic Potential in the Rat (aspartame).

E-89 (P.T. #1218S75), Evaluation of the Embryotoxic and Teratogenic Potential in Mouse (aspartame).

ISSUE 14:

A transcription error occurred in study E-5 which explains the lower incidence in the level of ossification of the cervical vertebral center observed in control rats which could not be explained by the petitioner in the submission to FDA.

COMMENT 14:

The number "83" was listed as the total number of control fetal skeletons with unossified cervical centrum. This number is actually the percentage of the control group found with unossified cervical centrum. The actual number of these fetal skeletons is 166. Calculations using the correct number as found in the raw data give a 82.8% incidence versus the 41.3% submitted. It should be pointed out, however, that the submission stated that the incidence in the level of ossification of the cervical vertebral centra in the treated animals (79.7% in low dose and 82.9% in the high dose) compared favorably with historical control data generated in this laboratory, and no meaningful explanation could be given for the low incidence (41.3%) seen in the controls in this study. Since the conclusion of the study was that there was no evidence of treatment-induced anatomical alterations, this change in percentage would not effect this conclusion. This noted error in transcription explains the anomaly reported for the controls; the controls were in fact as expected.

ISSUE 15 :

A physical inventory of the skeletal specimens (E-5) revealed that a total of 15 fetuses from the high dose group were missing (8%); no definite reason was given for the missing specimens. Additionally, the examinations of the visceral and skeletal specimens were not blind.

COMMENT 15 :

It would not appear that this loss of skeletal specimens was intended, or that this loss would affect the study. Searle's examination records correspond to what was reported to FDA. A certain amount of variation in findings normally occurs between individuals making these types of skeletal examination. Also, specimens of this type are fragile and tend to break-up. The inspection team teratologist examined the skeletal specimens that were available and found minor discrepancies which appear to be equally distributed among all dose levels. The animals in this study were numbered in numerical order with the controls having numbers 1-30, the low dose 31-60, etc. It is also probable that the specimens were stored in numerical order. Therefore, the possibility that specimens of the same dose group (8% of high dose) could be misplaced, is conceivable. Further, the method of numbering the animals allowed for the examiner to know the dose level of each animal. However, the protocol did not specify that these examinations were to be done blind.

ISSUE 16 :

Visceral examination of 329 specimens would appear (from the raw data) to have been performed in 2 days (02/27/70 and 03/05/70). No explanation was given by Searle personnel. This would be impossible for one person to accomplish.

COMMENT 16 :

The Searle scientist who did the examination estimated (via interview) that he examined approximately 30 fetuses per day, but the records do not indicate this.

It cannot be determined, from the available data, what these dates mean, or when the visceral examination was made.

ISSUE 17 :

The following skeletal findings were not reported in the submission to FDA on Study E-5:

1 - Hypoplasia of the maxilla observed in two low dose fetuses - this is a 1.1% incidence. The other groups did not show this anomaly.

2 - Sternum ossification - center split observed in one control - this is a 0.5% incidence.

3 - 3% upper, 1% lower incisors absent in the control; 4% upper, 4% lower incisors absent in the low dose group; 5% upper incisors absent in the high dose group.

COMMENT 17 :

These observations should have been reported; however, their omission does not appear to alter the data submitted.

ISSUE 18 :

All tissue slices from treated fetuses with anomalies in study E-5 were unavailable for examination. These had been destroyed prior to this inspection.

COMMENT 18 :

Only three anomalies were reported in the submission to FDA: hydrocephalus in one low dose and one high dose group, and hydronephrosis and hydroureter in one control animal. Blood was noted in the pericardial cavity of the visceral section of fetus 4601 and was marked "O.K." in the raw data. This was not listed in the submission. All other fetuses were marked "O.K." There were no sheets to specify the anomalies to be looked for. Some investigators have noted a 10% incidence in visceral anomalies in this species, while only a 1% incidence (all groups combined) in anomalies was observed in this study. Since there were no specimens to examine to authenticate the data recorded, no conclusion can be drawn as to the validity of these results as reported to FDA. This particular study was performed in 1970, and therefore it would not be unexpected that these specimens were no longer available. If they had been available, their usefulness would have been limited. It should be pointed out, however, that the raw data available for inspection is accurately reflected in the final

report; with the few exceptions noted above.

ISSUE 19 :

It was noted that Searle did not include abnormal findings of the visceral examination in its submission to FDA (E-89). Also, the findings in the 367 visceral sections examined pertained to only three fetuses (#20407, #32012, #41101). Two findings (#32012-cleft palate and 20407 - segmented uterus) were recorded in the raw data, verified by the FDA teratologist, but were not submitted to FDA. The FDA teratologist also noted a slight hydrocephalus of the ventrical and the enlargement was not in the raw data. The raw data further indicated that fetus #41101 had a "a renal pelvic cavitation of the kidney, not enlarged" and that it "is an artifact and not a malformation". The inspection team teratologist's examination indicated an enlarged renal pelvis with hydronephrosis. Another high dose fetus #41109 was examined by the inspection team teratologist; however, he was unable to locate the section made for the renal pelvic area. It should be noted that approximately 50% of the fetuses examined had one or more visceral sections that were too thick. Additionally, in several specimens (high dose level), not enough sections had been taken through the heart, and/or the renal pelvic area had been missed completely.

COMMENT 19 :

Instruction manuals for visceral exams are not specific with regard to the number of sections or thickness to be taken through the heart. The manuals available to the Searle examiner pertain primarily to rabbit and rat visceral examinations and not to the mouse. It should be noted that no abnormalities were observed in the control group. The submission stated that this strain has a less than 1% incidence in anomalies. There are no examination sheets that specify the abnormalities that are to be included in their examination of visceral sections. The Searle examiner of the visceral sections was more or less in charge of the whole experiment, and the investigators were unable to

determine the training and experience of this employee for this particular aspect of the study. While there is no evidence that the study was compromised by this issue, the practices of (1) examining sections which were too thick, and (2) not making enough sections through the organs, as specified in the protocol, does not preclude a possible failure to observe anomalies which may have occurred.

ISSUE 20 :

It was noted in the Searle submission to FDA that there was a significantly greater number of fetuses in the medium dose level with poorly ossified supraoccipital bones when compared to controls. The inspection team teratologist, therefore, examined the supraoccipital bones of fetuses in both the control and high dose level groups.

COMMENT 20 :

A certain amount of variation in findings normally occurs between individuals making these types of skeletal examination. A comparison of the % incidence of this anomaly found by the FDA teratologist and that reported in the submission data follows:

Group	Submission	FDA teratologist
Control	3%	4.5%
High dose	6%	8.5%

Although the FDA teratologist observed a higher percent incidence in this anomaly, both examiners observed a 50% difference in the incidence between control and high dose level (3%: 6% VS 4. 5%: 8.5%).

ISSUE 21 :

Individual skeletal sheets were not dated. It appears from the recordings on the reverse side of the laparotomy sheets that the skeletal examination of 500 fetuses occurred over a period of time (2 days, 5-19-75 and 6-4-75) which is insufficient for an accurate analysis of each fetal specimen. Further, the findings listed for each respective skeletal

fetus are for the most part incomplete because the research technician listed only the findings the examiner considered relatively unusual.

COMMENT 21 :

The submitted data, the raw data, and the observations by the FDA teratologist are virtually the same. It is probable that the data recorded on the laparotomy sheets might have been transcribed from another data source on two separate days. The FDA teratologists examined 5 litters/dose level and determined that the original skeletal exam records essentially agreed with the submission to FDA. The Searle examiner did not clearly differentiate between the total number of sternebrae centers that were absent and the total number of "small" sternebrae centers. However, the FDA teratologist verified their findings of major malformations. Although it is not clear when these examinations were made or the period of time in which they were made, the minor differences in classification of skeletal variations observed would be expected due to individual variations in classification. No serious errors were found.

ISSUE 22 :

There were no records to document the source and age of the male rats and mice used in these two studies (E-5 and E-89).

COMMENT 22 :

Documentation of the source and age of the males is important to determine whether they had previously been exposed to test substances which might effect the results of the study. However, the submitted report stated that the males were from a breeder colony maintained at the laboratory, and that the males were only used for breeding.

ISSUE 23 :

Premature deliveries were recorded for 2 mice (#236 and #308), but the pups were not weighed, measured, or sexed. Both appear to be full term. These pups were not used in any calculations made.

COMMENT 23 :

The FDA teratologist stated that it was probably a correct procedure to omit these pups because the plugs of the dams were probably missed, and hence the animals may have been dosed on incorrect days. In his opinion it would have been better if these litters had been examined, weighed, and the records kept. However, these omissions would not appear to invalidate the data.

ISSUE 24 :

Food consumption data in general were found to be in agreement with the submission. Five discrepancies were noted for 4 animals on different days.

COMMENT 24 :

These differences of one gram or less which were noted would not appear to affect the study. Four of the animals were in the low dose groups, and the increased amount consumed, as calculated by the investigators, is within the range of the amount consumed by others in this group.