FOR MEMBERS' USE ONLY

TOX/90/44

COMMITTEE ON THE TOXICITY OF CHEMICALS IN FOOD, CONSUMER PRODUCTS AND THE ENVIRONMENT

ASPARTAME - VALIDITY OF SEARLE TOXICITY STUDIES

Introduction

1. Members will recall that in December 1989 they discussed a submission by Dr E Millstone, University of Sussex which cast doubts on the validity of the original toxicity studies on aspartame carried out by the manufacturer, Searle (TOX/89/74 and TOX/MIN/89/7). A copy of the minutes of this discussion is attached at Annex 1. Although the Committee agreed that the COT had previously been correct to accept the FDA view that the toxicological conclusions from the Searle studies were valid, it decided to discuss this issue further after the Secretariat had obtained the documentation outlined in paragraph 31 of TOX/MIN/89/7. This has now been obtained from the FDA.

Chronology

2. The chronology of the relevant events is summarised in Table 1.

3. The FDA have sent copies of the 1977 Establishment Inspection Endorsements and Associated Reports (EIR) which resulted from an FDA Team's investigation of 3 studies on aspartame carried out from 25/4/77 to 8/7/77. The first EIR addresses the oral carcinogenicity study in the rat conducted on DKP, and describes problems with accurate recording of data and with the stability and homogeneity of DKP in the diet mixture. This EIR was seen by the Committee in TOX/89/7, annexed to the memorandum from the Bureau of Foods Task Force to HR Roberts, Acting Director, Bureau of Foods but in fact predates that memo. The second EIR discusses the rat and mouse teratology studies and

raises queries about the thoroughness of the visceral examination and of reporting of abnormalities to the FDA and about record-keeping.

4. The Secretariat now has a better copy of the memo to Dr Roberts, with the appendices referred to in the memo and this is attached at Annex 2. This memo reports the results of a Bureau of Foods Task Force whose remit was:

- to compare the findings in the EIRs for the three studies examined with the raw data contained in the exhibits and final reports

- 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

- 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.

Although the Task Force concludes that the studies are authentic, they uphold the concerns expressed about the adequacy of visceral examinations in the rat teratology study and homogeneity of diet in the DKP study. Indeed, it is rather surprising that this memo is considered by the FDA to resolve concerns about these studies.

5. The Committee has previously seen the summary and conclusions of the report of the audit carried out by UAREP (Universities Associated for Research and Education in Pathology) on 12 further 'pivotal' studies. The FDA has sent us a review of the UAREP reports by an FDA team (Annex 3). .Although clearly the UAREP report noted a number of discrepancies between raw data and reports, and in the diagnoses in histopathological and other examinations between Searle or contract house pathologists and UAREP pathologists, the conclusion of the FDA team review was that there were no discrepancies in any of the reports that were of sufficient magnitude or of a nature that would compromise the data

as originally submitted by Searle. This appears to be a reasonable conclusion from the data presented.

6. Subsequently, a Public Board of Inquiry was set up to help resolve the issues surrounding the proposed marketing of aspartame, particularly in relation to potential neuroendocrine effects and whether aspartame may induce brain tumours in the rat. The question of the validity of the Searle studies was not an issue. The Board of Inquiry recommended that aspartame should not be approved until further animal testing was conducted to resolve the brain tumour issue. However, this recommendation was overturned by the FDA Commissioner who published his final decision on aspartame in the Federal Register in July 1981, i.e. that the available data established the safety of aspartame for its proposed use. The only section of the Federal Register report which deals in any detail of the study validity question is attached at Annex 4 (Section vi Mr Turner's Appeal). The Commissioner argues that there was no need to address this issue again since the audits had confirmed that the studies were authentic and since no specific issues other than the one of DKP - diet homogeneity had been raised as evidence of poor conduct. On the homogeneity question, the Commission comments that the photograph which showed the DKP particles to be larger than the feed was taken of a sample prepared for stability testing purposes, not for use on test. "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 small amounts".

7. Aspartame was subsequently permitted for use in the US. In 1987 there was an investigation of the FDA's approval of aspartame by the US General Accounting Office, carried out at the request of Senator Howard Metzenbaum, a persistent critic of aspartame and of the US food additive approval process. The GAO staff were not scientists and did not address the scientific issues related to aspartame's safety. The report does deal at some length with the question of validity of the Searle studies. The relevant chapter is attached at Annex 5. The report accepts the FDA's actions in

authenticating the Searle studies were appropriate and that UAREP and the FDA Centre for Food Safety and Applied Nutrition (CFSAN) addressed the conduct of the studies in their investigation. However, it does not explain why the FDA considered some of these studies acceptable when there appears to be clear problems with their conduct.

8. Most recently, a Hearing was held before the Committee on Labour and Human Resources, United States Senate, on the Health and Safety Concerns of Nutrasweet on November 3, 1987. Senator Metzenbaum chaired the Hearing, which only lasted 4 and a half hours. The Secretariat has obtained a transcript of the Hearing (537 pages) which contains a large number of prepared statements and covers all the controversies, scientific and otherwise, concerning aspartame. The Committee has already seen the section of the hearing most relevant to the current issue - the statements of former FDA employees Dr J Verritt and Dr A Gross (see TOX/89/74), both of whom were very critical of the FDA's final decision regarding the adequacy of the Searle studies. We have located no other statements in the transcript which throw any more light on this issue.

Discussion

9. The additional documents obtained by the Secretariat tend to reinforce the conclusions which were derived from the documents submitted by Dr Millstone, i.e. that some of the Searle studies were very poorly conducted but that, despite this, the FDA concluded that they were adequate to be utilised in the safety assessment of aspartame. The studies about which there appears to be most doubt are the rat and mouse teratology studies on aspartame and the rat carcinogenicity study on DKP. Members will wish to note that a second rat teratology study was carried out using a 3:1 mixture of aspartame and DKP and a number of rabbit teratology studies were also conducted. An additional carcinogenicity study carried in Japan on a 3:1 mixture of aspartame and DKP and a mouse carcinogenicity study on DKP showed no adverse effects. In addition, last December the Committee

reviewed the main clinical and animal studies on aspartame reported since 1982 and concluded that they presented no cause for concern.

10. The Committee is now asked whether it stands by its previous decision to accept the FDA view that, despite the flaws in the Searle studies, the toxicological conclusions were unaffected, or whether it requires further studies on aspartame.

Secretariat
June 1990

TABLE 1

CHRONOLOGY

DATE	EVENT	DOCUMENTS
1973	Searle submitted a petition for aspartame's use in all foods	
July 1974	FDA approved aspartame's use in dry foods.	
July 1975	FDA Commissioner appointed a task force to investigate Searle's animal studies on 7 products, including aspartame. This on-site investigation was to determine if Searle submitted false information to FDA.	
December 1975	The task force concluded that some of the Searle studies were questionable. FDA stayed the aspartame regulation.	
July 1976	In response to the task force findings, FDA decided to investigate aspartame studies to determine whether FDA could rely on these studies to assess aspartame's safety.	
April 1977	An FDA team began investigating 3 aspartame studies.	Two establishment Inspection Reports*
August 1977	UAREP began investigating 12 further studies.	UAREP report* published November 1978
September 1977	FDA officials decide these 3 studies authentic.	Memo to HR Roberts 28/9/77.
March 1979	CFSAN concluded that the deficiencies found in both FDA and UAREP reviews were not significant enough to invalidate Searle's aspartame studies.	Memo to Dr N Singleton 16/3/79.

DATE	EVENT	DOCUMENTS
1980	Public Board of Inquiry held. Revoked aspartame's 1974 approval, concluding the more studies were needed to determine whether aspartame caused brain tumours.	
July 1981	FDA Commissioner's Final Decision on aspartame overturned PB01 decision and reapproved aspartame.	Published in Federal Register.
198? - 1987	General Accounting Office Investigation of FDA's approval of aspartame concluded it was satisfactory.	GAO report published June 1987.
November 1987	Senate Hearing (Metzenbaum enquiry)	Transcript.*

* Copies held by Secretariat. Copies of the relevant sections of the other documents cited are included in this paper.

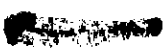
Annex 1 to TOX/90/44

Extract from TOX/MIN/89/7

[redacted]

28. [redacted] reminded Members that it was ten years since the toxicity data on aspartame were first examined. It had been apparent at that time that there were some questions about the validity and general standard of the data. Searle were found to fallen short of the required standards and major changes in laboratory practices had since been instigated. The COT had, however, been assured that the data presented were adequate to make an assessment.

29. [redacted] explained that the submission from Dr Millstone (TOX/89/74) was not concerned with whether aspartame was toxic but with whether the original studies were so seriously flawed that they should not have been relied upon when making the original decision on aspartame. The COT had in the past taken a positive view based on the FDA stance that the studies, although flawed, could be relied upon from a toxicological viewpoint. The FDA's view had been reported orally to the COT in July 1979 by the then



Action

Medical Secretary, [redacted]. Although the Department then had copies of the UAREP report, the Secretariat had no record of the Committee seeing the written FDA report, part of which formed annex 2 to TOX/89/74. [redacted] asked members if the information in annex 2 was of sufficient concern for them to request that the studies were repeated. [redacted] pointed out that the Millstone document was likely to receive considerable publicity and that the Secretariat should be given a clear indication of the COT's views on the matter.

30. Members agreed that although the FDA and UAREP investigations had revealed a large number of errors and flaws in some of the studies, the COT had been correct to accept the FDA view that the toxicological conclusions were unaffected. It was noted that in pre-GLP days regulatory authorities sometimes had to rely on data which would not be considered adequate by current standards when making decisions. The alternatives to not accepting the FDA view were discussed. The Committee noted that requesting further studies on aspartame would require changing its status from Group A to Group B. The possibility of asking the DH GLP Unit to perform an audit was discussed but dismissed on the grounds that the FDA's own audit had been exhaustive.

31. Members did, however, consider that documents which had not so far been seen by the Secretariat or the Committee should be obtained and reviewed. The Secretariat was asked to obtain a transcript of the Senate hearings and the background to them, a written copy of the FDA's conclusions that the flawed studies could be used for assessment of aspartame, and a copy of the FDA inspector's report to Dr Howard, Director of the Bureau of Foods in 1977. It was agreed to defer any further discussion until these documents had been obtained.

Secretariat

[redacted]

[redacted]

Annex 2 to Tox/90/44

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

[redacted]

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 Tumorigenicity 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.

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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	8.42×10^6/CMM	8.28×10^6CMM
E10MM (day 734)	RBC	8.20	10.12		
E15LM (day 734)	RBC	34.00	7.73	11.85×10^6/CMM	7.55×10^6CMM
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 ($p \leq 0.05$)

#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'a 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.

	Treatment Day					
Group°	42	92	189	364	546+	735+
Control			mean, (mg/DL)			
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			
	Range	Average	Accord		w/Protocol	Percentage
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 DKP, 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 G25LM 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 #40109 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.

Annex 3 to Tox/90/44

MEMORANDUM

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

TO : Dr. Neal Singletary
Team Leader: Bureau of Foods: Aspartame
Petitions Control Branch (HFF-334)

DATE: March 16, 1979

FROM : Dr. Charles J. Kokoski [redacted] 3/16/79
Chief, Food Additives Evaluation Branch (HFF-185)

SUBJECT: Review of the UAREP Authentication Report on Aspartame Studies.

This memo serves as transmittal cover memo of the team review of the UAREP Authentication Report submitted to the Bureau of Foods on December 13, 1978.

The members of the FDA Team who evaluated the UAREP Report are:

Dr. Richard Kraska/HFF-334 [redacted]
Dr. Patrick Siu/HFF-185 [redacted]
Dr. Neil Sass/HFF-190 [redacted]
Dr. Linda Taylor/HFF-335 [redacted]
Dr. Morris Weinberger/HFF-130 (Auxilliary Member)
Dr. Prem N. Dua/HFF-130 (Auxilliary Member)
Dr. Dennis Ruggles/HFF-110 (Auxilliary Member)
Dr. Charles J. Kokoski/HFF-185 (Team Leader)

Individual members were assigned specific studies to review independently in the first phase of the effort. They were assisted by auxilliary member from Division of Pathology/HFF-130 and Division of Mathematics/HFF-110 as the needs arose.

Following the first phase, the team as a whole met frequently to review and critique each individual evaluation. The package attached represents the final product of the team effort in evaluating the UAREP Report.

The following studies of G.D. Searle's Food Additive Master File 134 were subjects of the UAREP Authentication Report:

E-70:	Lifetime feeding study in the rat
E-11:	2-generation reproduction study in the rat
E-28:	106-week feeding study in the dog
E-33 & 34:	2-year feeding study in the rat
E-90:	Teratology study in the rabbit
E-75:	104-week feeding study in the mouse

MEMORANDUM

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

TO : Charles J. Kokoski, Ph.D. DATE: March 9, 1979
Chief, Food Additives Evaluation Branch, HFF-185

FROM : Patrick M.L. Siu, Ph.D. PS. 3/12/79
Food Additives Evaluation Branch, HFF-185

SUBJECT: Authentication Review of Selected Material Submitted to the Food and Drug Administration Relative to Application of Searle Laboratories to Market Aspartame prepared by Universities Associated for Research and Education in Pathology, Inc. (UAREP) dated November 8, 1978.

This memo is in response to your oral request (see also HFF-185 memo of conference dated 1/2/79) that I answer the question of whether the discrepancies, if any, noted by UAREP in the authentication of the toxicity data in Studies E-33, 34, E-70 and E-87 would compromise these studies. The earlier toxicity studies under review were those performed by EPL (subcontractor of Hazleton Labs, HLA) and Dr. Innes (Searle's neuropathology consultant) and submitted with the petition on aspartame. This assignment did not involve the reevaluation of the toxicity data by EPL and Dr. Innes nor does it involve the evaluation of the data by UAREP.

The present attached reports include the reviews of UAREP authentication of the following:

1. Attachment I - Re: Study E-33, 34 (SC-18862, P-T No. 838H71). Two-Year Toxicity Study in Rat.

2. Attachment II - Re: Study E-70 (SC-18862, P-T No. 892H72). Lifetime Toxicity Study of Aspartame in the Rat.

3. Attachment III - Re: Study E-87 (P-T Nos. 838H71 and 892H72). A Supplemental Study of Rat Brains from Two Tumorogenicity Studies.

The observations and conclusions in light of the UAREP report, FDA Division of Pathology and Division of Mathematics evaluations (see attached memos) are as follows:

E-76: 110-week feeding study of DKP in the mouse

E-87: Supplemental study of rat brain

E-86: Supplemental study of dog brain

E-9: Neonate rat study

E-19: Endocrine study

E-88: Monkey study

The Team review of this report concerned itself with the basic question: whether or not any discrepancies between the data originally submitted and the data as authenticated would be sufficient to compromise the results of the study. When opinions of protocol design and evaluation of data were expressed by UAREP in its report, the team did not necessarily comment on these matters, in that they were not issues of authentication of data originally submitted to FDA.

Although UAREP noted some discrepancies in the Aspartame data, the summation of the team review of the UAREP report may be stated as: there were no discrepancies in any of the reports that were of sufficient magnitude or of a nature that would compromise the data as originally submitted by the G.D. Searle Co. petitioner of Aspartame.

I thank the team members and auxilliary members for their diligent and timely effort in providing a comprehensive review.

cc:with attachments:

cc: HFF-109
HFF-152 (Flamm, Edwards)
HFF-150 (Blumenthal)
HFF-144
HFF-185 (Siu, Kokoski)
HFF-190 (Sass)
HFF-334 (Kraska)
HFF-335 (Taylor)
HFF-110 (Ruggles)
HFF-130 (Weinberger, Dua)
FAP# 3A-2885

CJKokoski:dlm:3/9/79:HFF-185:472-5767

1. For Study E-33, 34:

   a. UAREP noted few minor errors in transcription, computation and rounding-off of numbers.
   b. Histopathological findings by UAREP agree in general with those of EPL and Dr. Innes.
   c. There is nd reason to believe that the discrepancies noted by UAREP would alter the recommendation derived based on the toxicity data of EPL and Dr. Innes.

2. For Study E-70:

   a. In general, good agreement was found by UAREP with the early toxicity data of EPL and Dr. Innes.
   b. The discrepancies noted by UAREP would not compromise Study E-70.

3. For Study E-87:

   a. The discrepancies noted by UAREP in brain tumors of Studies E-33, 34 and E-70 combined would not compromise Study E-87.

Attachments: I. Study E-33, 34: II. Study E-70; III. Study E-87.
Memos: HFF-134 memo dated 2/28/79; HFF-110 memo dated 3/2/79.

cc:HFF-100
HFF-152

FMLSiu:klr:3/9/79:HFF-185:472-5767

ATTACHMENT I - Review of UAREP Authentication of Study E-33, 34 (SC-18862, P-T No. 838H71). Two-Year Toxicity Study in Rat.

CRcd rats (40M & 40F/test group, 60M & 60F/control group) were fed aspartame at the 0, 1 (low dosage), 2 (medium dosage), 4 (high dosage) and 6-8 g/kgbw/day (very high dosage) in the diet. The very high dosage group received 6 g/kgbw/day for the first 16 wks, followed by 7 g/kgbw/day to wk 44, and then 8 g/kgbw/day for the remainder of the 104 wk experimental period.

Results:

Palpable tissue masses and nodules: UAREP reported 13/40 tissue masses or nodules in males given the low dose whereas Hazelton Labs (HLA) reported 7/40. Very little difference between UAREP's and HLA's findings was noted in the other test groups and the controls. On 38 occasions tissue masses or nodules were reported as present at one of the designated observation intervals (6, 13, 26, 52 and 104-wk) and then not observed at subsequent intervals could be expected in that many of these lesions could have been missed by subsequent examinations especially by multiple observers.

Body weight changes: UAREP agreed with HLA's data.

Food consumption: UAREP's findings agreed with HLA's.

Compound consumption: A computational error indicated that the test animals received about 1/3 of the designated dosage for days 3 & 4 of the experiment. This discrepancy is of no consequence to the 104-wk study.

Survival: UAREP noted that the survival time (mean value) for females at the very high dosage group was 602 days, whereas that reported by HLA was 423 days. The control group (females) was 659 days by UAREP and 657 days by HLA. Thus, the survival time of 602 days noted by UAREP is actually closer to the control values of 659 days (UAREP) and 657 days (HLA).

Clinical Lab studies: UAREP found no significant discrepancies between the lab notebooks and the submitted data. No transcriptional errors were noted for hematology and blood chemistry.

Urinalysis:UAREP found no transcriptional errors.

Opthalmoscopic examination: UAREP found no discrepancies.

Non-neoplastic diagnoses: Agreement in diagnoses by EPL and UAREP was remarkably good. There were, however, discrepancies in total numbers of specific diagnoses which could indicate differing inclinations of pathologists to make these diagnoses. For instance, in the spleen, EPL diagnosed extramedullary hematopoiesis in 66% of all rats, i.e., 9 times as frequently as UAREP, but seldom diagnosed reticuloendothelial cell hyperplasia which was reported 25 times more by UAREP. EPL diagnosed

Histopathologic findings: UAREP's findings were in general agreement with EPL's. FDA Division of Pathology (HFF-134 memo dated 2/28/79) stated that the discrepancies between UAREP and EPL in terms of brain tumors were not significantly different and concluded that these discrepancies do not change the previous conclusions expressed in their memo dated 11/7/78 entitled "Pathology Review of Aspartame....."

FDA Division of Mathematics (HFF-110 memo dated 3/2/79) indicated that the findings of UAREP and Dr. Innes are essentially the same. The differences were that UAREP found a brain tumor in the male control group not originally reported by Dr. Innes, and Dr. Innes had reported on brain tumor in the males at the 1 g/kg/day group, which was UAREP's findings were less significant statistically than Dr. Innes', which indicates that the discrepancies noted by UAREP would not compromise Study E-33, 34.

Conclusions:

1. UAREP noted only a few minor errors in transcription, computation and/or rounding-off of numbers.

2. Histopathologic findings by UAREP agree in general with those by EPL and Dr. Innes.

3. The discrepancies noted by UAREP would not have altered the decision made earlier on Study E-33, 34 based on the submitted data of EPL and Dr. Innes.

ATTACHMENT II - Review of UAREP Authentication of Study E-70 (SC-18862, P-T No. 892H72).Lifetime Toxicity Study of Aspartame in the Rat.

CRcd weanling rats (40M & 40F/test group, 60M & 60F/control group) were given aspartame at 0, 2 and 4 g/kgbw/day dose levels for 104 wks. The parents of these weanling rats were pretreated with aspartame for 60 days prior to mating. Thus, these test rats were exposed to the compound throughout organogenesis, gestation and lactation periods. The exposure of the compound was from the day of conception for 104 wks.

Results:
Palpable nodules, tissue masses or skin lesions: UAREP found only minor discrepancies which do not affect the validity of the study.

Body weight changes: Of the 24 body weight mean and 24 standard deviation values, there were 33 complete agreements and only one varied more than 5%. UAREP findings thus were not significantly different from the earlier submitted data.

Food consumption: Minor variations were noted by UAREP, but were not critical in terms of the overall results of the experiment. Of the 47 comparisons, 44 of the UAREP determinations were within 2% of the HLA's report and 31 of the 44 were identical.

Survival data: The mean survival time at the 104 wk as computed by UAREP agreed completely with HLA for the control and the low dose groups of males with no statistically significant differences in survival between male or female rats fed aspartame at either dosage level or their respective controls.

Clinical lab studies: Neither transcriptional nor computational errors were noted by UAREP for the hematology and clinical chemistry parameters.

Urinalysis: UAREP's report did not reveal any discrepancies.

Ophthalmoscopic examination: No apparent discrepancies were noted by UAREP.

Necropsy: UAREP noted only 5 inconsequential errors of rounding-off of numbers and one minor computational discrepancy out of 96 mean values and 96 standard deviations.

Histopathologic findings: There was generally close agreement in the numbers of tumors in each category analyzed by UAREP and EPL. UAREP noted the presence of adrenal cortical and medullary and pituitary tumors for which EPL did not look. However, the differences between the control and two test groups were not significant.

Division of Pathology (HFF-134 memo dated 2/28/79) concluded that there were no significant differences in the diagnoses of brain tumors by UAREP, EPL and Dr. Innes.

FDA Division of Mathematics (HFF-110 memo dated 3/2/79) found no significant increases in brain tumors in treatment groups over the control groups. Thus, the discrepancies noted by UAREP were minor.

Conclusions

1. In general, good agreement was found by UAREP with the earlier data of EPL and Dr. Innes.

2. The discrepancies noted by UAREP would not compromise Study E-70.

ATTACHMENT III - Review of UAREP Authentication of Study E-87 (P-T Nos. 838H71 and 892H72). A Supplemental Study of Rat Brains from Two Tumorogenicity Studies.

At the time of the initial EPL review, there were no brain sections made on study E-33, 34 rats in groups 2 (low dosage, 1 g/kgbw/day), 3 (medium dosage, 2 g/kgbw/day), and 4 (High dosage, 4 g/kgbw/day), and no sections on group 2 rats in study E-70, unless such brains showed gross lesions. Subsequently, the brains were sectioned on all rats and reviewed by Dr. Innes, but he only reported on tumors of glial and meningial origin. Both EPL's and Dr. Innes' diagnoses were included with the petition on aspartame. The discrepancy between UAREP's and Dr. Innes' findings on brain tumors was mainly in the description of the lesions. As UAREP had pointed out, this discrepancy "undersores the difficulty of absolute identification of individual gliomas without the aid of special techniques that often include elaborate histologic stains and electron microscopy." A notable discrepancy existed in the diagnosis of meningioma by Dr. Innes and EPL in one female rat in group 3 in Study E-70, since no lesion was observed in the slides presented to UAREP. UAREP indicated that it was possible that they did not have the same slide as that reviewed by Dr. Innes. FDA Division of Pathology (HFF-134 memo dated February 28, 1979) found no significant difference in the incidence of primary brain tumors between the control and any of the treated groups based on the UAREP findings and concluded that there were no major differences in the diagnoses of brain lesions reported by EPL, Dr. Innes and UAREP. This memo further states that the discrepancies noted by UAREP, do not change the conclusions reported in their previous memo dated 11/7/78 entitled "Pathology Review of Aspartame..." that the brain neoplasms in these studies occurred spontaneously and had no relationship to treatment with aspartame.

FDA Division of Mathematics (HFF-110 memo dated 3/2/79) did not include statistical analysis on the combined data from studies E-33, 34 and E-70 because these two long-term studies were performed under different conditions. It could be inferred from the considerations of Studies E-33, 34 and E-70 that the combination of these two studies, assuming that it were proper to combine them, would also suggest that the discrepancies noted by UAREP will not compromise the study E-87.

Conclusion:

1. The discrepancies noted by UAREP on brain tumors in studies E-33, 34 and E-70 would not compromise study E-87.

MEMORANDUM

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

TO : Dr. Charles J. Kokoski
Chief, Food Additives Evaluation Branch HFF-185

DATE: February 28, 1979

THRU : Morris A. Weinberger, M.D. [redacted]
Director, Division of Pathology HFF-130

FROM : P.N. Dua, D.V.M., Ph.D. [redacted]
Petition Review Pathologist HFF-134

SUBJECT: Division of Pathology Comments on UAREP Neuropathology Consultant's Review of Rat Brains for E-33, 34 and E-70 (Pathology Project No. P-27).

This is in response to your request for our comments on the above cited subject matter.

A copy of page 838, Table 9-1 from volume 3 of the UAREP report containing all of the brain neoplasms diagnosed by EPL, Innes and UAREP is attached. Based upon this Table, we prepared a summary Incidence Table of primary brain tumors (Table 1, attached).

Of the 18 animals listed in Table 9-1 (Section E-33, 34), EPL examined 8, Innes examined 13 and UAREP examined 18. In Section E-70, of the 10 animals listed, EPL and UAREP examined all 10 and Innes examined 9. Also, as indicated in Table 9-1, we note the following discrepancies in diagnoses between the three reviewing groups:

Path. No.	EPL Diagnosis	Innes Diagnosis	UAREP Diagnosis
E-33,34			
64-603	no lesion	no lesion	astrocytoma
64-777	---*	astrocytoma with ependymal components	no lesion
64-713	---	ependymoma	astrocytoma
64-715	---	ependymoma	astrocytoma
64-926	ependymoma	ependymoma	astrocytoma
64-652	meningioma	----	no lesion
64-881	meningioma	sarcoma (meningeal)	medulloblastoma/ meningeal sarcoma
64-900	meningioma	----	meningoencephaliti
E-70:			
71-660	metastatic carcinoma	----	no tumor
71-686	meningioma	meningioma	no lesion

* Indicates brain sections were not examined initially

The above described differences in diagnoses between EPL, Innes and UAREP are also reflected in minor changes in the total number of primary brain tumors as indicated in Table I.

The most frequent discrepancy was the interpretation of the lesion reported by EPL and Innes as ependymoma, which UAREP reported as astrocytoma. Although astrocytoma and ependymoma represent different types of cells in the brain, both are considered in the group of gliomas. Path. No. 64-881 was reported as meningioma, meningeal sarcoma and medulloblastoma/meningeal sarcoma by EPL, Innes and UAREP, respectively. These differences, we feel, are a matter of different pathologic interpretations of the slides by the reviewing pathologists.

In the Path. Nos. where lesions were reported by one group and "no lesion" found by the other group, it is possible that the groups did not review the same slides.

Since UAREP was selected by both Searle and FDA to review all suspected brain lesions in all treatment groups and controls and because of their widely recognized expertise in diagnostic pathology, we feel that it is proper to utilize their findings in the principal analysis of the data. The brain tumor data as reported by UAREP, in our opinion, do not appear to be related to treatment, dose or sex. By using a basic two-tail chi-square test (programmable Canon calculator model No. 167 P-II), we found no significant difference in the incidence of primary brain tumors between the control vs any of the treated groups.

In summary, we feel that there were no major differences in the diagnoses of brain lesions reported by EPL, Innes and UAREP. The discrepancies in diagnosis that are reported could be attributed to the individual interpretations by the three groups of the morphological appearance of tumors using a variation in terminology. These discrepancies, in our opinion, do not change the conclusions reported in a previous memo addressed to you from this office (dated 11/7/78, entitled, "Pathology Review of Aspartame..."; that the brain neoplasms in these studies occurred spontaneously and had no relationship to treatment with aspartame.

Should you have any questions, please call us at 245-1123.

cc: Dr. Patrick Siu HFF-185
Central File HFF-130

(b)(6)

Prem N. Dua, D.V.M.,Ph.D.

Attachments:

(b)(6)

Morris A. Weinberger, M.D.

TABLE I

Total Tumor Incidence of Primary Brain Tumors in Entries --33,34 and E-70

Group Nos.	Aspartame Levels	EPL Diagnosis			Innes Diagnosis b			UAREP Diagnosis		
E-33,34	(g/kg/day)	Male	Female	Total	Male	Female	Total	Male	Female	Total
I	0	0/58	--	--a	0/59	0/59	0/118	1/59	0/59	1/118
II	1	--	--	--	2/36	2/40	4/76	1/36	2/40	3/76
III	2	--	--	--	1/40	0/40	1/80	1/40	0/40	1/80
IV	4	--	1/4	--	4/39	1/40	5/80	4/39	1/40	5/80
V	6-8	1/40	3/39	4/79	0/40	2/38	2/77	0/40	2/38	2/77
E-70										
I	0	3/60	1/57	4/120	3/58	1/57	4/115	3/58	1/57	4/115
II	2	2/39	1/39	3/80	2/36	1/39	3/75	2/36	1/39	3/75
III	4	1/39	1/40	2/80	1/40	1/40	2/80	1/40	0/40	1/80

a It is indicated that no brain sections were examined at the time of initial EPL review

b For incidence of tumors in Innes diagnosis, we used the same denominators as in UAREP for comparison purposes only.

Table 9-1

CARCP Neuropathology Consultant's Review of Rat Brains for E-33,34 & E-70

Group	Animal No.	Path No.	EPL Diagnosis	Innes Diagnosis	CARCP Diagnosis
E-33,34					
1M	83-651	64-603	no lesion (d)	no lesion (d)	astrocytoma
2M	83-745	64-775	---	astrocytoma	astrocytoma
2M	83-750	64-777	---	astrocytoma with ependymal components	no lesion
2F	83-769	64-989	---	astrocytoma	astrocytoma
2F	83-766	65-011	---	astrocytoma with ependymal components	astrocytoma
3M	83-837	64-764	---	astrocytoma with ependymal components	astrocytoma
3F	83-861	64-977	---	---	involved by pituitary carcinoma
4M	83-888	64-712	---	oligodendroglioma	oligodendroglioma
4M	83-892	64-713	---	ependymoma (d)	astrocytoma
4M	83-895	64-715	---	ependymoma (d)	astrocytoma
4M	83-919	64-707	---	astrocytoma with ependymal components	astrocytoma
4F	83-934	64-926	ependymoma (d)	ependymoma (d)	astrocytoma
5M	83-995	64-662	meningioma (d)	---	no lesion
5F	84-007	64-979	no lesion (d)	---	involved by pituitary carcinoma
5F	84-010	64-881	meningioma (d)	sarcoma (meningeal)	medulloblastoma/meningeal sarcoma
5F	84-019	64-898	astrocytoma/glioma	glioma, unclassified	astrocytoma
5F	84-033	64-900	meningioma (d)	---	meningoencephalitis
5F	84-034	64-901	no lesion (d)	---	involved by pituitary carcinoma
E-70					
1M	90-818	71-465	astrocytoma	astrocytoma	astrocytoma
1M	90-819	71-466	astrocytoma	astrocytoma	astrocytoma
1M	90-838	71-502	astrocytoma	astrocytoma	astrocytoma
1F	90-876	71-635	astrocytoma	astrocytoma	astrocytoma
1F	90-927	71-660	metastatic carcinoma (d)	---	no tumor
2M	90-943	71-581	ependymoma	ependymoma	ependymoma
2M	90-967	71-600	glioma/astrocytoma	astrocytoma	astrocytoma
2F	90-969	71-701	glioma/astrocytoma	astrocytoma	astrocytoma
3M	91-016	71-526	astrocytoma	astrocytoma	astrocytoma
3F	91-061	71-656	meningioma (d)	meningioma (d)	no lesion

(d) indicates significant discrepancy with CARCP diagnosis.

At the time of initial EPL review, there were no brain sections on E-33,34 rats in groups 2, 3, and 4, and no sections on group 2 rats in E-70, unless such brains showed gross lesions. Subsequently, the brains were sectioned on all rats and reviewed by Dr. Innes, but he only reported on tumors of glial and meningeal origin.

MEMORANDUM

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

TO : Dr. Patrick Siu
Division of Toxicology
HFF-185

DATE: March 2, 1979

FROM : Dennis Ruggles
Division of Mathematics (HFF-110)

SUBJECT: Statistical Reveiw of Brain Tumors in Rats Given Aspartame for 2 Years

The Division of Mathematics was given Table 9-1 and Appendix IX-3 which contained data on brain tumors in rats given aspartame over a 2 year period. There were two studies, E-33, 34 and E-70, involved in the analysis. In the E-33, 34 study the rats were given aspartame at 0, 1, 2, 4, and 6-8 g/kg. In the E-70 in-utero study the rats were given the compound at 0, 2, and 4 g/kg. Of interest was the comparison of the Innes and UAREP diagnoses of brain tumors for the same rats.

A Fisher's Exact test (one-tail) was used to compare the treated groups and the control for the proportion of rats with brain tumors. For the UAREP diagnosis there was a borderline significant ($p=0.085$) increase in brain tumors over the control at the 4 g/kg level for males, while for males and females combined there was a significant ($p=0.040$) increase over control at the 4 g/kg level. For the Innes diagnosis no brain tumors were found in the male control group as opposed to one in the UAREP diagnosis. Also one additional brain tumor was found at the 2 g/kg level in the Innes diagnosis. Thus, when control males were compared to 4 g/kg males there was a significant ($p=0.024$) increase in brain tumors. When males and females are combined there is a significant increase in brain tumors at the 2 g/kg level ($p=0.025$) and at the 4 g/kg level ($p=0.010$). See Table 1.

For the E-70 study the results of both the Innes and UAREP diagnoses were in close agreement. No significant ($p>0.10$) increases in brain tumors over control were found at any treatment level.

Dennis Ruggles

(b)(6)

attach:

cc: HFF: 100
HFF: 110 r/f
DR/lmk

Table 1: Brain Tumors in Rats Given Aspartame, Study E-33, 34

Dose:	0		1		2		4		6-8 g/kg	
UAREP Diagnosis	#with #animals	%	#with #animals	%	#with #animals	%	#with #animals	%	#with #animals	%
Males	1/59	1.7	1/36	2.8 p>0.20	1/40	2.5 p>0.20	4/40	10.0 p=0.085	0/39	0 p>0.50
Females	0/59	0	2/40	5.0 p>0.10	0/40	0 p>0.40	1/40	2.5 p>0.20	2/40	5.0 p>0.10
Males + Females	1/118	0.8	3/76	3.9 p>0.10	1/80	1.2 p>0.20	5/80	6.2 p=0.040	2/79	2.5 p>0.10
Innes Diagnosis Males	0/59	0	2/36	5.6 p>0.10	1/40	2.5 p>0.20	4/40	10.0 p=0.024	0/39	0 p>0.40
Females	0/59	0	2/40	5.0 p>0.10	0/40	0 p>0.40	1/40	2.5 p>0.20	2/40	5.0 p>0.10
Males + Females	0/118	0	4/76	5.3 p=0.022	1/80	1.2 p>0.20	5/80	6.2 p=0.010	2/79	2.5 p>0.10

MEMORANDUM

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

TO : Charles J. Kokoski, Ph.D.
Division of Toxicology, HFF-185

DATE: March 8, 1979

FROM : Richard C. Kraska, Ph.D. (b) (6)
Division of Food and Color Additives, HFF-334

SUBJECT: Evaluation of the Authentication Review by UAREP (Chapter XI) of the Two-Generation Reproduction Study in Rats (E-11) on Aspartame

This two generation study was designed to evaluate and characterize the effects of aspartame at 2 g/kg and 4 g/kg on the reproductive performance of albino Charles River cesarean-derived rats.

UAREP stated in their report that the incidence of discrepancies or problems they encountered was less in this study than in most of the others selected for UAREP review.

Some of the minor discrepancies found by UAREP were the following:

1. Lack of behavioral observations.

The protocol for this study specified that the behavior of the animals was to be observed during the course of the study. UAREP could not find any records for any behavioral observations made during the study.

Conclusion: Lack of recording such possible observations does not compromise the study.

2. Diet Consumption Data.

UAREP criticized the recording of the amount of food consumed by the animals in this study. The only information concerning food consumption sent to UAREP gives means and ranges of consumption at weeks four and nine. UAREP is of the opinion that data recorded should have been more comprehensive and that food consumption should have been measured more often.

HLA did report that the animals in the test groups consumed less of the ration than desired by the protocol. HLA estimated from the consumption data that the first parental generation and second parental generation at worst consumed 12% and 23%, respectively, less food than anticipated at

times during the course of the study. UAREP reanalyzed the consumption data and by pro-rating changes in body weight with the food consumption data they estimate as much as 25% less food may have been consumed by the first parental generation and 38% less food may have been consumed by the second parental generation than anticipated during the third week after weaning of these animals. UAREP estimates that the largest deviations in consumption of the diet occurred during the third week after weaning.

Conclusion: These potential differences in food consumption are considered inconsequential.

3. Statistical Analysis of body weight data.

HLA reported statistically significant reductions from controls in the mean litter weights in the high-dose group only. HLA did not statistically analyze the parental weights. UAREP did not find any discrepancies in the raw data for the body weights. UAREP did reanalyze the data using their usual battery of statistical tests (ANOVA, LSD, Q and t-tests). They found the mean body weights of some low-dose groups were statistically significant from the control group in some tests and not in others. In the reanalysis of the statistics for the body weights for the high-dose groups, UAREP found that in all comparisons to the control group reported to be statisically different by HLA, that significance was indicated by all four statistical tests calculated by UAREP.

Conclusion: The statistical conclusions on some of the observations in the low-dose group are at best equivocal. These observations do not compromise the quality of the study.

4. Reproductive indices

UAREP validated and agreed with HLA data on fertility, gestation, live birth, and lactation indices.

Conclusion: No problem.

CONCLUSION

The discrepancies found by UAREP do not compromise the report on Study E-11.

MEMORANDUM

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

TO : Dr. Charles J. Kokoski
Chief, Food Additives
Evaluation Branch (HFF-185)

DATE: February 10, 1979

FROM : Dr. Neil Sass, Colors & Cosmetics [(b)(6)]
Evaluation Branch (HFF-190)

SUBJECT: E-28 106-Week Oral Toxicity Study In the Dog

This study was conducted by Searle Laboratories under the designation P-T No. 855570 using Aspartame admixed with a diet of Purina Dog Meal which was supplemented with Wayne Lab-Blox and water. Animals were divided into dosage groups receiving 0.0, 1.0, 2.0, or 4.0 g/kg of Aspartame with normal human exposure anticipated to maximize at 30 mg/kg/day. Five male and five female beagles were included in each dosage group. The control animals received a dietary supplement which approximated the caloric intake furnished by the Aspartame in the test diet at the medium dose level (2.0 g/kg). The initial protocol was amended several times with respect to feeding schedules to achieve a more realistic and viable method of obtaining daily food and compound consumption data. Interim progress reports were submitted periodically through the course of the 106-week study.

Discrepancies noted by UAREP.

A. Apparent lack of randomization of littermates between treatment groups, and age and weight discrepancies between animals entered into the study.

In the conduct of a research protocol, randomization is utilized to reduce any effects which may be inherent in the genetic background of the animals used. This becomes problematical when conducting studies involving dogs from a facility-operated colony. The ability to produce usable litters (same parturition date, litter size, weight, etc.,) within a reasonable time frame may be difficult. It is also inherent in the definition of randomization, that animals not be identified prior to group selection. Only through the use of a non-random technique could the appearance of multiple animals from a single litter in specific groups have been prevented.

Conclusion: The selection of animals in a random fashion, resulting in multiple animals from a single litter being included in a single group, was not detrimental to the outcome of the study nor did the imbalance of initial body weights/group elicit significant negative effects.

B. Discrepancies in the recording of body weight data

As body weight constituted only one parameter evaluated during the course of this study, and since any drastic change would have been expected to be substantiated by abnormal data from other clinical parameters monitored, the toxicological significance which could be ascribed to an apparent change in body weight alone is minimal.

Conclusion: Since no toxicologically significant abnormalities in clinical parameters were associated with the abnormal body weight data, the discrepancies were seen to create no problems with respect to the outcome of the study.

C. Discrepancies involving haematological analyses.

UAREP has cited discrepancies, primarily related to Coulter Counter obtained haematological data through the course of this study. The use of mechanical means of determining blood parameters can be fraught with inherent variances not detectable at the time of analysis in spite of the use of standardization procedures. The differences encountered by Searle and UAREP appear to be functions of individual daily machine variances. No time or dose-related trends appeared in the data presented by UAREP, thereby negating the toxicological significance of this data.

Conclusion: The abnormal appearance of haematological data did not indicate either dose-related or time-related effects and as such, imply analytical variability which falls within the realm of normal occurrence. These discrepancies, therefore, would not have a significant impact on the interpretation of the results of this study.

Conclusion: In the absence of discrepancies in the reporting of histopathological findings and the minimal numbers of discrepancies associated with the reporting of other parameters monitored, there is no reason to assume that the report on Study E-28 as presented by Searle is compromised in any way.

MEMORANDUM

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

TO : Charles J. Kokoski, Ph.D.
Division of Toxicology, HFF-185

DATE: March 8, 1979

FROM : Richard C. Kraska, Ph.D. [redacted]
Petitions Control Branch, HFF-334

SUBJECT: Evaluation of the Authentication Review by UAREP (Chapter XIV) of the Embryotoxic and Teratogenic Potential of Aspartame in the Rabbit (E-90)

This study was designed to evaluate the embryotoxic and teratogenic potential of aspartame and its hydrolysis products, L-phenylalanine and L-aspartic acid, in the pregnant rabbit. Aspartame at 0.5, 1.0 and 2.0 g/kg was administered by gavage during fetal organogenesis (gestation 6-18 days).

Sacrifice was at gestation day 28. Fetuses were weighed, measured and examined grossly prior to further fixation. Half the fetuses of a litter were fixed and preserved in Bouin's solution for examination by the free-hand razor blade sectioning technique of Wilson. The remaining half of each litter was preserved in 95% aqueous ethanol before evisceration and processing of the skeletons by the Alizarin Red S staining and clearing technique.

A summary of the problems found by UAREP is as follows:

1. Food consumption data

In 237 instances (3%) out of 8400 food consumption entries, UAREP found that the Entry Book reported "ND = no data" for food consumption while the raw data showed "negative consumption" or rather a gain in weight of the food container during a twenty-four hour period. There were 309 instances (4%) in which the Entry Book reported "ND" and UAREP found data. UAREP has no explanation for either situation, although it is recognized that rabbits tend to eat their own feces instead of food.

Statistically lower food consumption was observed in the high dose group and was noted in the original FDA evaluation (Collins, September 11, 1978) UAREP made a total of 3024 statistical comparisons on the food consumption data. These new statistics confirm a generalized decrease in food consumption during the entire period of administration of the high dose of aspartame. The new statistics do suggest a sporadic decrease in food

consumption for the low and medium doses but no decrease which persisted through the entire period of administration.

UAREP similarly scrutinized the body weight data and made new statistical comparisons, but did not find any consistent new effects.

Conclusion: These discrepancies do not compromise the study.

2. Review of the Skeletal Specimens

UAREP found ten discrepancies in the diagnosis in their review of the 801 skeletal specimens preserved from the study. In seven of the ten cases, Searle did list these animals as malformed and UAREP either disagreed with the diagnosis or found additional anomalies besides those reported. In two of these seven cases, UAREP felt there was no malformation where Searle felt there was. In the three other specimens, UAREP found supernumerary nasal sutures, which Searle did not report. Of these three animals, one was in the control group, one in the low dose and one in the medium dose aspartame groups.

Conclusion: The discrepancies found by UAREP do not compromise the study.

3. Review of body cross sections

UAREP did attempt to re-examine the soft tissue specimens, but discontinued the task since due to the poor conditions under which they were stored. Because of the poor specimens, they were concerned about potential inaccuracies in any comparisons with the original data.

The UAREP report states that "the examination of the 801 skeletal specimens is adequate to evaluate the accuracy of the Searle teratological diagnosis."

Conclusion: The problem encountered by UAREP does not compromise the study.

CONCLUSION:

UAREP did not find any new information which invalidates the study submitted as E-90.

MEMORANDUM

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

TO : Dr. Charles J. Kokoski, (HFF-185)
Chief, Food Additives Evaluation Branch

DATE: 7 MAR 1979

FROM : Dr. Linda L. Taylor, (HFF-335) (b)(6) [redacted]
GRAS Review Branch

SUBJECT: E-75; 104 Week Oral Toxicity Study of Aspartame in the Mouse

This study (P-T No. 9Q4H73) was contracted with Hazleton Laboratories America (HLA) and was designed to evaluate and characterize the toxicity of aspartame to weanling mice fed 1, 2, and 4 grams aspartame per kilogram body weight per day for 104 weeks. The 360 mice used in this study were divided into control groups of 72 males and 72 females, and three treatment groups, each containing 36 males and 36 females. Data were collected on clinical observations, body weight changes, food and compound consumption, survival, opthalmoscopic observations, hematologic and clinical laboratory studies, tumor incidence, necropsy, and histopathological observations. The original protocol was amended five times. This experiment was orginally planned as an 80-week study, but was extended to 104 weeks, although sacrifice began at 103 weeks.

There were many discrepancies noted by UAREP in this study, but none of these, alone or in combination, was found to invalidate the results reported by Searle.

Summary of major discrepanices noted by UAREP:

1- Discrepanices in mean survival times and in percent survival.

The mean survival time (days) differences cannot be explained; however, the number of days found by UAREP does not alter the result that no difference was noted in mean survival time. The discrepancies in the number of days are distributed evenly throughout the groups.

The differences in percent survival involve 5 or fewer mice per group, with the usual number being 2 or less. The order of increasing survival for males found by Searle was: high, medium, low, control; by UAREP: medium, low, high, control. The order for the females was: Searle- low, all other groups equal; for UAREP- medium, high, low, control. Neither analysis shows a statistical difference in percent survival.

CONCLUSION: The differences noted in survival between Searle and UAREP would not have a significant impact on the interpretation of the results of the study.

2- Some confusion regarding termination plans.

The original protocol was for an 80-week study. This was amended to extend the study to such time as 25% of the control male or female group remained. Actual survival of mice was determined by HLA at 100 weeks but terminal sacrifice was carried out during the 103rd week. The title of the study and many of the tables indicate this is a 104 week study.

UAREP stated that data did not show a significantly lower incidence of death in any one of the groups of mice that would result from determining survival to 100 instead of 103 weeks. Although equal numbers of mice from each group were not sacrificed on the same days, a one week difference out of 103 weeks in the date of sacrifice would not be expected to produce any significant distortion in the results.

CONCLUSION: The discrepancies noted would not affect the interpretation of the results.

3- Discrepancies involving hematology and clinical chemistry data.

UAREP found various parameters to be statistically significantly different at various time periods, but these were not reported to be different by HLA. Additionally, UAREP commented on the small sample size and the wide variability of the values found among individual mice within the same group (all values were reported to FDA). UAREP stated that this could obscure small but significant biological variation due to treatment and that the variability of the data makes it worthless.

CONCLUSION: All values were known to FDA, including sample size and range of values within groups (and trends could be observed). There were no dose-related or time-related effects. These discrepancies, therefore, would not have a significant impact on the interpretation of the results of the study.

4- Discrepancies in tumor incidence probability.

UAREP and HLA values for the analysis of tumor incidence probability do not agree closely. However, there was generally good agreement between the data up to the intervals of 90 and 100 weeks, with poorer agreement in the terminal weeks. For the study, there was no statistical or biological evidence that aspartame had tumorigenic properties.

CONCLUSION: The discrepancies noted do not compromise the study.

5- Discrepancies in histopathologic diagnosis.

Of the significant discrepancies noted by UAREP, approximately 25% would be major, of which half would relate to difference between a benign tumor and a proliferative hyperplasia. The discrepancies showed no significant increase in any of the groups of experimental mice. The discrepancies noted in diagnosis are what one would expect to observe between two trained pathologists.

CONCLUSION: The discrepancies noted do not affect the conclusion that, under the conditions of the study, there is no evidence of any disease predominantly in any of the experimental groups.

6- Discrepancies involving phenylalanine determinations.

All surviving mice were to be sampled; however, only 31 mice were. Additionally, only termination samples were taken. UAREP questions analysis of phenylalanine only at termination in light of the statement in the HLA project sheet that evaluation of serum PA levels provides evidence for compound absorption, and questions whether there was any significant absorption of aspartame. UAREP analysis of the data also showed no statistical difference which agrees with the findings of HLA. However, if the male and female groups are combined for each group to increase the sample size, the high dose group is statistically significantly increased over the control.

CONCLUSION: All of the values were known to FDA and could have been calcuated- the trend was evident. Therefore, the discrepancies noted would not affect the interpretation of the results.

CONCLUSION: In the absence of discrepancies in the reporting of the various parameters measured, and in the lack of any statistically significant differences in tumor incidence, there is no reason to assume that the report on Study E-75 as presented by Searle is compromised in any way.

MEMORANDUM

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

TO : Dr. Charles J. Kokoski, (HFF-185)
Chief, Food Additives Evaluation Branch

DATE: 7 MAR 1979

FROM : Dr. Linda L. Taylor, (HFF-335) (b)(6)
GRAS Review Branch

SUBJECT: E-76; 110 Week Oral Toxicity Study of Diketopiperazine in the Mouse

This study (P-T No. 98H71) was contracted with Hazleton Laboratories America (HLA), and was designed to evaluate and characterize the toxicity of the in vitro conversion product of aspartame, diketopiperazine (DKP) to weanling mice fed 0.25, 0.5, and 1.0 grams DKP per kilogram body weight per day for 110 weeks. The 360 mice used in this study were divided into control groups of 72 males and 72 females, and three treatment groups, each containing 36 males and 36 females. Data were collected on clinical observations, hematologic and clinical laboratory studies, tumor incidence, necropsy, and histopathological observations. The original protocol was amended eight times. This experiment was originally planned as an 80-week study, but was extended to 110 weeks, although terminal sacrifice began at 108 weeks.

There were many discrepancies noted by UAREP in this study, but none of these, alone or in combination, was found to invalidate the results of the study as reported by Searle.

Summary of major discrepancies noted by UAREP:

1- Discrepancies in tumor incidence probability.

UAREP and HLA values for the analysis of tumor incidence probability do not agree closely. However, there was generally good agreement between the data up to the intervals of 90 and 100 weeks, with poorer agreement in the terminal weeks. On the basis of UAREP's analysis of the data collected, there was no evidence that DKP was tumorigenic under the conditions of this study.

CONCLUSION: The discrepancies noted do not compromise the study.

2- Discrepancies in survival analysis.

Although there was generally good agreement in the HLA and UAREP life table analysis data up to the intervals of 90 or 100 weeks, there was generally a poorer agreement in the terminal weeks. The differences noted between HLA and UAREP cannot be explained completely; however, the number of days found by UAREP does not alter the result that no difference in survival was found between any of the groups. The differences are evenly distributed among the groups. The differences in percent survival involve 3 or fewer mice per group. HLA noted a statistically significant difference in the survival of the medium dose females. This was not confirmed by UAREP.

CONCLUSION: The discrepancies noted in the survival data would not have a significant impact on the interpretation of the results of the study.

3- Discrepancies in histopathological diagnoses.

Of the significant discrepancies noted by UAREP, only 20% are major discrepancies of non-neoplastic lesions, and an additional 17% are significant in that they involve the difference between a benign tumor and hyperplasia. Considering the large number of microscopic sections involved, this does not represent an impressive list of discrepancies, and there is no predilection for such discrepancies in any one group compared with another. Additionally, the differences noted are what one would expect to observe between trained pathologists.

CONCLUSION: The discrepancies noted do not affect the conclusion that, under the conditons of the study, there is no evidence of any disease predominantly in any of the experimental groups.

4- Variability in hematologic results and in clinical chemistry determinations.

Many of the clinical chemistry determinations were performed on only 2 mice, and the results within groups covered a wide range. UAREP stated that the data would not be of any use. All of the values were known to FDA and, therefore, were available during the original evaluation.

CONCLUSION: The minor discrepancies noted would not have any impact on the results of the study.

CONCLUSION: In the absence of discrepancies in the reporting of the various parameters measured, and in the lack of any significant differences in tumor incidence, there is no reason to assume that the report of Study E-76 by Searle is compromised in any way.

MEMORANDUM

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

TO : Dr. Charles J. Kokoski
Chief, Food Additives
Evaluation Branch (HFF-185)

DATE: February 10, 1979

FROM : Dr. Neil Sass, Colors & Cosmetics (b)(6)
Evaluation Branch (HFF-190)

SUBJECT: E-86 A Supplemental Study of Dog Brains from a 106-Week Oral Toxicity Study

Searle undertook this supplemental study of brain tissues from beagles involved in E-28 in order to ascertain the significance of some of the findings initially reported in E-28. Additional blocks of brain tissue were sectioned in an attempt to determine whether the diagnosis of minimal, focal, subependymal proliferation of glial cells in the third ventricle was, in fact, the true interpretation of the presentation of these tissues. In addition to a review of these slides by Microscopy for Biological Research (Searle) and UAREP, a panel of consultant neuropathologists was also convened by UAREP to obtain an expert opinion. All slides were read in a blind fashion with pathologists being unaware of the dose, to include controls, groups to which the animals from which the tissue was derived were assigned. The evaluation was concerned with the possible presence of neoplastic lesions, inflammatory processes, vascular alterations, demyelinating states, or disturbances in the number, architectronics, or cytologic appearance of neurons in these tissues.

Conclusion: The results of the reassessment of cerebral tissues from animals which had previously been diagnosed as possessing abnormal pathology indicated that no untoward or compound-related discrepancies could be associated with the feeding of aspartame to beagles.

MEMORANDUM

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

TO : Charles J. Kokoski, Ph.D.
Division of Toxicology, HFF-185

DATE: March 9, 1979

FROM : Richard C. Kraska, Ph.D. [redacted]
Division of Food and Color Additives, HFF-334

SUBJECT: Evaluation of the Authentication Review by UAREP (Chapter X) of the Toxicological Study of Aspartame on Neonatal Rats (E-9)

UAREP reports "This study was performed at Hazleton Laboratories America (HLA Project 700-241) with rats from F_{2A} litters of the Two-Generation Reproduction Study discussed in Chapter XI. This project was designed to evaluate and characterize the effects of aspartame on hematological and biochemical parameters as well as on tissues of rats one through 21 days of age."

There were three treatment groups: control, low dose (2g/kg) and high dose (4g/kg). Twenty male and twenty female pups were in each group. Five pups of each sex from each group were sacrificed at 24 hours and 5, 15 and 21 days postpartum. The test compound was available in the diets at the levels indicated to the maternal animals. The mother's milk was never analyzed for aspartame or its metabolities. Pups were also exposed in utero since mothers were eating aspartame from the test diets during pregnancy. Pups may have eaten some of the diet between days 15 and 21 as they approached the weaning age of 21 days. Histology was only done on heart, liver, kidney, stomach and urinary bladder.

UAREP mainly addressed two issues in this study in their report: (1) white blood cell counts and (2) the kidney histology and possible pathology. UAREP also documented the mis-sexing of 3 pups. UAREP sympathizes "Sexing of newborn rats is difficult."

White Blood Cell Data

UAREP found that the white blood cell counts reported for the day 5 sacrifice by HLA were not corrected for nucleated red blood cells. Nucleated red cell counts were made by HLA, these counts were recorded and the white cell counts were corrected accordingly in the laboratory worksheets. Due to an apparent transcribing error, however, only the uncorrected values were recorded in the entry books and used in the final report and in the statistical tests.

UAREP has provided the corrected and uncorrected white cell counts for the day 5 sacrifice (Table 10-1). Although UAREP specifically states this was a problem for day 5 (p. 849) UAREP does not mention in the text whether the data from the other sacrifices were corrected for nucleated red cells. However, in Table 10-2, UAREP summarizes the statistical tests for the corrected and uncorrected white cell counts for males at day 15 and females at day 21, noting that HLA did not statistically analyze the corrected data. The only possible reason HLA did not statistically analyze these data would seem to be that a similar error in transcribing the data to the entry books occurred for the day 15 and 21 sacrifices as occurred for the day 5 sacrifice.

Conclusion: Despite the discrepancies found by UAREP and the unclearness in the report by UAREP on the status of the white cell data for the day 15 and day 21 sacrifices, we conclude that these discrepancies are inconsequential.

Kidney Histology

Regarding the renal pathology, UAREP generally agreed with the original diagnoses and extent of findings.

In the FDA evaluation of this study (Memorandum of August 31, 1973), the transient nature of the pathology in this study was noted. In a related study of 28-30 day postpartum rats treated similarly, no such treatment-related kidney abnormalities were seen. Although UAREP critized the quality of the sections for the 28-30 day old rats, UAREP generally agreed with EPL's assessment.

Conclusion: No serious discrepancies were noted by UAREP.

CONCLUSION

UAREP did not uncover any new information about this study that should change the FDA position that a review of E-9 is not essential for a decision about the safety of aspartame.

MEMORANDUM

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

TO : Dr. Charles J. Kokoski
Chief, Food Additives
Evaluation Branch (HFF-185)

DATE: February 10, 1979

FROM : Dr. Neil Sass, Colors & Cosmetics [redacted]
Evaluation Branch (HFF-190)

SUBJECT: E-19 A Sweetening Agent: Endocrine Studies

Searle conducted seven hormone-related and six physiologic response tests as a routine battery for compounds undergoing pre-clinical testing. This submission comprised reports from the battery conducted during aspartame trials. Animals utilized included albino mice, Sprague-Dawley rats, rabbits, hamsters, and C57B1 mice. Aspartame and DKP had been administered to the test species intragastrically to simulate the human route of exposure. Exposures to the test compounds ranged from 1 hour to 7 days with the exception of the pituitary gonado trophin assays which ran for 14 days, androgenic-myotrophic studies which ran for 20 days, and chronic polyarthritis assay which ran for 19 days.

Discrepancies noted by UAREP included the inability of UAREP reviewers to interpret or recalculate statistical analyses presented in the Searle reports concerning immunosuppressive activity or antiinflammatory activity.

Conclusion: The lack of a valid interpretation of the data in which discrepancies noted by UAREP were reported would not compromise the outcome of the report. The type of data which is subject to question is data which is unrelated to the proposed use of Aspartame, and any effects which may have been observed in these short-term assays would have become more manifest under chronic study conditions.

MEMORANDUM

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

TO : Charles J. Kokoski, Ph.D.
Division of Toxicology, HFF-185

DATE: March 5, 1979

FROM : Richard C. Kraska, Ph.D. (b)(6)
Petitions Control Branch, HFF-334

SUBJECT: Evaluation of the Authentication Review by UAREP (Chapter XII) of the Toxicological Study of Aspartame on Mated and Pregnant Rhesus Monkeys; A Compilation of Available Fragmentary Data (E-88)

Validation of this study was not requested by FDA nor was this study critical to our safety evaluation.

E-88 was done in the same laboratory as E-32, the 52 week infant monkey study. After the principal investigators death, records were found which indicated female monkeys other than those in E-32 were fed aspartame for a period. This data was submitted as E-88.

The data for E-88 is indeed fragmentary and no conclusions of safety or hazard can be made about aspartame from this information. Not all the females became pregnant, not all pregnancies reached term, not all pregnancies which reached term resulted in a live birth and not all neonates survived for more than a few days. Since only 8 females total were used in the study statistical analysis of this data is fruitless. Moreover, daily doses and lengths of exposure were not standard and the females used were not suitable specimens for a reproduction study since they were socially maladjusted. Neither the original report of E-88 nor the UAREP review of this study find any evidence that these animals experienced any seizures, which was the major toxicity found in the study with infant monkeys. UAREP also mentions these same monkeys may have been used for other studies during the course of their lifetime.

CONCLUSION

UAREP did not find any new information which should alter our decision that this study is inconsequential to the decision on the safety of aspartame.

Annex 4 to TOX/90/44

(d) *Conclusion on E-70.* For the reasons stated above, I consider E-70 to be a negative study.

4. *E-77/78* (Vols. 89-90) [40]—a. *Study Design.* This study differed from E-33/34 and E-70 in that the test compound used was diketopiperazine (DKP), a breakdown product of aspartame (less than 2%) (Vol. 112 at 30-31). Charles River CD (Sprague-Dawley) albino rats were also used in this study. Three treatment groups, consisting of 36 rats per sex per group, were fed DKP as part of their regular diets at dosage levels of 0.75, 1.5 and 3.0 g/kg body weight/day for 115 weeks, beginning after weaning. A control group of 72 animals per sex was fed the same diet without DKP. Test animals were sacrificed at the end of the dosing period, and their brains (seven sections per animal) were examined histologically.

b. *Study results.* The Board reported the following results and offered the following evaluation:

> In the E-77/78 study concerning the diketopiperazine of aspartame 5 tumors were recorded: 2 in the control group of 123 rats (1.6%), and the remaining 3 among the 158 animals of the three experimental groups (1.5%). Two of the 5 gliomas could have been noted on gross inspection of the brain.
>
> This study shows no difference between experimental and control groups, and the recorded percentages fall within the high range of normal incidence reported from various normative studies.

(Board's Decision at 43)

c. *Analysis of and Conclusion on E-77/78.* None of the hearing participants challenge this interpretation of the data. Accordingly, I agree with the Board that E-77/78 is a negative study.

5. *Additional evidence: The Japanese study* (Searle's Exceptions, Appendix 2). This study was conducted only recently by the Japanese firm Ajinomoto Co., Inc., and concluded after the Board issued its decision. A preliminary report was submitted by Searle as part of its exceptions. I have considered this study as evidence in this proceeding, acknowledging that neither the Board nor the hearing participants have formally commented on it.

The preliminary report at (page 1) contains the following summary:

> The brain tumorgenicity of aspartame (APM) and of its diketopiperazine (DKP) was studied in 860 SLC Wistar rats. APM at dietary levels of 1 g/kg, 2 g/kg, 4 g/kg or APM+DKP (3:1) 4 g/kg was fed for 104 weeks. One atypical astrocytoma was found in a control rat and 2 astrocytomas, 2 oligodendrogliomas and 1 ependymoma were scattered among the 4 test groups. There was no significant difference in the incidence of brain tumors between control and test groups. It is concluded that neither APM nor DKP caused brain tumors in rats in this study.

Taking the available information at face value, this appears to be a negative study in terms of brain tumors. Without a review of the Bureau of Foods, however, as well as by other interested parties, I do not believe it proper to base approval of aspartame on this study's results. Nor is such a course necessary in this instance. The three chronic studies discussed above (E-33/34, E-70, and E-77/78) are sufficient for me to make a final determination on the safety of aspartame in terms of its potential for brain tumors in rats. However, because the Japanese study suggests that aspartame does not cause brain tumors in a second strain of rat, the SLC Wistar rat, this study provides additional support for my conclusion on the brain tumor issue.

D. Conclusion on Brain Tumor Issue

For all the reasons stated above, I conclude that the available data, taken as a whole, establish that there is a reasonable certainty that aspartame and DKP do not cause brain tumors in laboratory rats. This conclusion is based on studies E-33/34, E-70, and E-77/78, all of which were considered at the hearing. Additional support for this conclusion is found in the Japanese study, submitted by Searle after the Board issued its decision. Accordingly, under the act's general safety clause, I find that the available data establish the safety of aspartame, in terms of brain tumors, for its proposed use.

VI. Mr. Turner's Appeal

Mr. Turner and Dr. Olney have repeatedly challenged the quality of data produced in Searle's animal studies. Indeed, Mr. Turner has petitioned for the Public Board of Inquiry to be reconvened because of the Board's refusal to consider what he called the "scientific validity" of the studies [41] (Vol. 153, Tab 187; see also Turner's Exceptions).

The Board disagreed with Mr. Turner's characterization that it failed to consider the "scientific validity" of the studies, asserting that the Board "did not exclude evidence relating to the quality or appropriateness of the experimental design of the studies or the scientific conclusions that can validly be drawn from the studies" (Board's Decision at 7). What the Board did decline to do was to "undertake a retrospective quality inspection of all the studies presented to it" which the Board considered had already been accomplished by UAREP and FDA (*id.*). Quite clearly, the Board considered its charge, as delineated in the June 1, 1979 Federal Register statement, to relate only to interpretation of the data and not conduct of the studies.

Both Searle and the Bureau agreed with the Board's ruling on Mr. Turner's appeal (Searle's Reply to Turner's Appeal, Vol. 157, Tab 200 and Searle's Reply to Turner's Exceptions; Bureau's Reply to Turner's Appeal, Vol. 157, Tab 208; and Bureau's Reply to Turner's Exceptions).

I believe the problem is partly one of semantics, as the phrase "scientific validity" may have several different meanings. The Board understood Mr. Turner to mean that it should redo UAREP's work which was to authenticate the data (i.e., make sure that the studies were actually conducted). Clearly, the board was correct in not attempting to repeat UAREP's work. The Board, in turn, uses the term "scientific validity" to mean the conclusions that can be drawn from the data presented, including study design. These conclusions were clearly within the Board's domain, and it was based on these considerations that the Board reached its ultimate findings. There is a third area, however, that lies somewhere between those two. This relates to the *manner* in which the studies were conducted. Even if the studies were not fraudulent, that does not necessarily mean that they were well conducted. A non-fraudulent study might be conducted in such a poor manner that its results would not be considered meaningful (*cf.* 45 FR at 61478, col. 2). As then FDA Chief Counsel Richard A. Merrill wrote to Mr. Turner on February 24, 1977, questions regarding the "execution of the studies" could be raised at the public hearing (Attachment No. 1 to Turner's Appeal, Vol. 153, Tab 187).

I conclude, however, that a new hearing need not be held. With one exception discussed below, Mr. Turner has not stated with particularity any deficiencies in the conduct of any of the pertinent studies which he believes, either alone or collectively, are sufficiently serious as to warrant a

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[40] This study was not reviewed by Dr. Innes. Neither was it reviewed by UAREP, although a similar authentication was performed by the agency (vol. 151, Tab 187).

[41] Mr. Turner's full prayer for relief included:

1. An order directing the Board to reconvene and consider whether certain studies have been validated;
2. An order directing an additional Board or other public investigatory body to validate these studies; and
3. Withholding of aspartame's approval until such validation is completed.

(Vol. 153, Tab 187 at 24-25)

study's invalidation.[42] Rather, Mr. Turner's (and Dr. Olney's) main criticisms appear to be mere speculations which fail to raise any genuine issue of material fact.

For example, Mr. Turner and Dr. Olney rely heavily on the 1976 Congressional testimony of then Commissioner Alexander M. Schmidt who characterized Searle's animal laboratory practices as "sloppy" (Tr./III/page 129, lines 1–4). That testimony was based on findings of an FDA investigation of two of Searle's *drug* studies which only peripherally concerned aspartame. The relevance of this investigation to the aspartame proceeding is that it triggered the detailed audit conducted by UAREP and the agency, and therefore, for the purposes of this proceeding the drug study investigation was superceded by the UAREP/FDA audit. Nevertheless, based on the "sloppy" laboratory practices theory, Dr. Olney attributed the slightly higher incidence of brain tumors found in the E–70 control animals over concurrently treated animals to a hypothetical mix-up that may have occurred between the control and treated groups (Olney's Pre-Hearing Position Paper, Vol. 151, Tab 160, Part III at 15). The speculation inherent in this allegation was evidenced at the hearing when, as the issue of the higher control incidence in the E–70 animals arose, the following exchange took place:

> Dr. Spitznagel [Consultant to Dr. Olney]: Our only comment on that is we have our suspicions, mainly that some of the controls were actually treated.
>
> Dr. Bussey [Consultant to Searle]: Do you have evidence to that effect?
>
> Dr. Spitznagel: No, we really don't other than the assertion of the Commissioner of FDA.
>
> (Tr./III/page 242, lines 20–25).

The only specific allegation by either Mr. Turner or Dr. Olney relates to the E–77/78 carcinogenicity study conducted on DKP. Dr. Olney cites a Bureau of Foods report that raises the possibility that the DKP-containing feed may not have been homogeneous (Report from Bureau of Foods' Task Force, September 29, 1979, pages 10–11, Volume 151, Tab 167). Dr. Olney's point here is that the non-homogeneous feed may have resulted in the "treated" animals' selectively not eating the DKP.

The Bureau of Foods' documents at issue relate to the authentication review conducted by FDA.[43] The pertinent documents were placed into the record by the Bureau shortly after the hearing, at the request of Dr. Olney and Mr. Turner (Volume 151, Tab 167). The documents include portions of FDA's on/site inspection report of Searle as well as a Task Force memorandum interpreting and commenting on that report.

The agency's investigation culminated in a Bureau Task Force Report which thoroughly discussed the homogeneity issue. The Task Force concluded that, although the homogeneity issue could not be conclusively resolved, no serious problems were encountered which would invalidate the study. The remedy advocated by the Bureau, and adopted by the agency, was to notify Searle by letter of laboratory practices which should be corrected in the future (see Memorandum for the Files, dated September 26, 1977 prepared by Taylor M. Quinn, and draft letter to Searle from Commissioner Kennedy (undated), both in Vol. 151, Tab 167).

Dr. Olney's one piece of "hard evidence" was a photograph of a feed mixture showing DKP particles larger than that of the feed, so that the animals in the treated group might have discriminated in favor of the smaller non-DKP particles (photograph attached to Olney letter of February 6, 1980, Vol. 151, Tab 165).

I agree with the Bureau that the evidence is not sufficient to invalidate this study. The photograph in question was taken by a sample prepared especially for stability testing purposes, not feeding purposes. As the Task Force wrote: "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" (Vol. 151, Tab 167, Task Force Report, Appendix A at 10–11). Thus, Dr. Olney's allegation here also appears to be speculative.

Nor is it necessary to order a new validation of these studies, as Mr. Turner suggests. Although the UAREP audit was undertaken to determine whether the aspartame studies were authentic or fraudulent, the three volume report covering over 1,000 pages contain detailed observations of how these studies were conducted.

UAREP has addressed itself to the question of whether the experiments were *carried out according to protocol plans* and the accuracy and reliability with which the experiments were performed and reported to the FDA. (Vol. 110 at 2) (emphasis added). Indeed, UAREP addressed such issues as: (1) Protocols; (2) clinical observations; (3) body weight, food, and compound consumption; (4) survival data; (5) clinical laboratory studies; (6) ophthalmoscopic observations; (7) necropsy; and (8) histopathology (Vol. 110 at 5–15) as well as (9) personnel, facilities and methods; (10) animals and animal care; and (11) data production, handling and storage (Vol. 110 at 20–22). The FDA portion of the audit had a similar scope. These are very similar subject areas to those which Mr. Turner raises in his appeal (see Vol. 153, Tab 187 at 14–15). Yet, not once does Mr. Turner cite examples from the UAREP report as evidence of poor conduct of the studies. His request for a new "validation" review, therefore, appears to be merely a fishing expedition for evidence of "sloppy" laboratory practices.

It should be emphasized that UAREP, a consortium of nine universities, has unquestioned expertise in the area of preclinical animal testing and that its review of Searle's studies was undertaken with complete neutrality. Although UAREP, like the agency, noted some procedures and irregularities that warrant improvement, none were of such a serious nature as to invalidate an entire study. Indeed, UAREP noted, and I agree, that review of the histopathologic slides provides a better basis for validation of the data than many of the other parameters (Vol. 110 at 23). On this point UAREP noted general agreement between its pathologists' reviews and the original diagnoses (*id.* at 24–25). UAREP also noted that both Searle and Hazelton Laboratories were accredited by the American Association for Accreditation of Laboratory Animal Care which, at the time, carried out the most through and critical nationwide evaluation of animal care facilities (*id.* at 20).

Therefore, based on the extensive information available in the record regarding the conduct of Searle's studies, and Mr. Turner's failure to raise with particularity any specific issues other than the one discussed above, Mr. Turner's appeal is denied.

VII. Conditions of Use

The third issue at the hearing was defined as follows:

> Based on answers to the above questions,
>
> (a) Should aspartame be allowed for use in foods, or, instead should approval of aspartame be withdrawn?
>
> (b) If aspartame is allowed for use in foods, i.e., if its approval is not withdrawn, what

[42] Mr. Turner has had ample opportunity to do so, either at the hearing, as part of his "appeal" submitted after the hearing, or as part of his exceptions filed after the Board's decision.

[43] E–77/78 was one of the three studies which FDA, rather than UAREP, audited (see Section I above).

Annex 5 to Tox/90/4

GAO

5502'

United States General Accounting Office

Report to the Honorable
Howard M. Metzenbaum, U.S. Senate

June 1987

FOOD AND DRUG ADMINISTRATION

Food Additive Approval Process Followed for Aspartame

GAO/HRD-87-46

Chapter 3

FDA Concluded Problems Did Not Invalidate Studies' Results

In approving aspartame or other food additives for marketing before 1975, FDA relied on the integrity of the manufacturer to submit reliable safety data. This integrity was questioned while FDA prepared for the public hearing requested by the objectors to aspartame's approval. In July 1975, FDA's Commissioner established a task force to review certain Searle animal studies, including those relating to aspartame. Preliminary results of this investigation raised questions about the accuracy and reliability of the data that CFSAN evaluated to establish aspartame's safety. As a result, FDA prevented Searle from marketing aspartame and in 1976 decided to conduct a more detailed investigation of 15 aspartame studies.

An FDA team investigated 3 of the studies and UAREP investigated the other 12 to determine if Searle submitted accurate and reliable data to FDA. In addition, the FDA team and UAREP considered how well the studies were conducted in reaching overall conclusions on each study. Although problems were found with the studies, CFSAN concluded it could use the studies as a basis to establish aspartame's safety. We believe the UAREP and the FDA team's investigations and CFSAN's evaluation of the aspartame studies were responsive to the 1975 task force findings on the aspartame studies. However, we did not evaluate the scientific issues raised or the adequacy of FDA's resolution of these issues.

FDA Found Problems With Searle Studies

In 1974 and 1975, FDA investigators identified problems with animal studies for two Searle drugs already marketed. Following this discovery, the FDA Commissioner appointed a task force to investigate animal studies supporting seven Searle products, including aspartame. The task force identified problems with the studies. As a result of the task force findings, the Commissioner placed a stay on FDA's approval of aspartame, preventing Searle from marketing it, and the Department of Justice instituted grand jury proceedings against Searle based on the findings in the animal studies for one drug product.

FDA Established a Task Force to Review Searle's Studies

On July 23, 1975, FDA's Commissioner appointed a task force because FDA investigators found problems with Searle's laboratory practices and inaccurate reporting of tumor findings on two marketed drugs, Flagyl and Aldactone.[1] For example, certain types of tumors noted on raw data entries of Flagyl tumor studies were unaccountably changed. Also,

[1]Flagyl is used to treat infections. Aldactone is an antihypertension drug.

Searle had submitted to FDA an incomplete report on the number of tumors seen in animals who had been given Aldactone.

The Commissioner directed the task force, composed of FDA pharmacologists and investigators, to review Searle's practices in conducting animal experiments and to determine if Searle submitted false information to FDA. The task force was to recommend appropriate regulatory actions based upon its findings. The task force selected for review 25 animal studies that supported seven products: the food additive aspartame and six drugs, including Flagyl and Aldactone. The task force considered for selection any drugs or food additives on which Searle had performed animal studies since 1968. They gave higher priority to products to be used over a long period of time and to those with a potential to cause tumors. Since aspartame was a food additive, it had highest priority based on the large number of people expected to use it over a long period.

In selecting animal studies for investigation, the task force gave higher priority to long-term animal studies, because such studies were potential indicators of long-term health effects that were not necessarily monitorable in humans. Moreover, these studies involved more animals, more observations, more record keeping, and more personnel. The task force selected 11 aspartame studies for investigation; Searle performed 9 and contracted with Hazleton Laboratory and the University of Wisconsin for the other 2 (see app. II for a list of these 11 studies).

Task Force Identified Problems That Prevented Searle From Marketing Aspartame

The task force found that many of the problems with the aspartame animal studies, as well as the drug animal studies, resulted from of a lack of quality control. For example, Searle technicians did not keep accurate and consistent reports on the animals' condition. In addition, protocols (written plans for a scientific experiment) were not followed in carrying out the studies. According to the task force members, without adequate control of every step of a study, one could not assess the adequacy of the results.

Based on preliminary task force findings, in December 1975, the FDA Commissioner placed a stay on the July 1974 aspartame approval, preventing Searle from marketing this product. Also, CFSAN had the task force seal the supporting data relating to the aspartame studies at Searle and Hazleton Laboratories until it could determine what actions to take.

The task force concluded that its investigation had uncovered evidence that Searle's practices were in violation of the Federal Food, Drug, and Cosmetic Act. They said "the results were so serious in some studies as to make it difficult, if not impossible, to draw conclusions regarding the full toxic potential of the products from the data." (See app. III for the task force's findings and CFSAN's comments.)

The task force report issued in 1976 recommended that

- the Department of Justice institute grand jury proceedings against Searle,[2]
- FDA establish regulations outlining good laboratory practice,[3] and
- FDA centers determine whether to take administrative and/or regulatory actions on each of the Searle products investigated.

CFSAN Concluded Aspartame Studies Were Reliable

Based upon the task force findings, CFSAN decided to perform a more detailed investigation of 15 aspartame studies to determine their accuracy and reliability by comparing Searle's data with the data in reports submitted to CFSAN. However, CFSAN lacked sufficient resources to perform such a review and believed it should select a group of scientists, independent of FDA and Searle. FDA asked Searle to contract with UAREP, a group of university pathologists. However, an FDA team began reviewing 3 of the 15 Searle studies. Four months later, UAREP began reviewing the other 12 studies.

FDA Scientists and University Pathologists Selected to Review 15 Studies

Before the 1975 task force, FDA relied on the integrity of the manufacturer to submit reliable safety data in supporting petitions for food additives such as aspartame. However, as a result of the task force findings, the FDA Commissioner stated the integrity of the submitted data supporting FDA's original approval of aspartame was questionable. He recommended a review mechanism that, "operating independently of but funded by Searle or other private sources, would promptly undertake to validate pre-selected studies that comprised the basis for [the] original approval of aspartame."

[2]The information collected on the drug studies served as a basis for convening a grand jury investigation of Searle. Searle was not indicted.

[3]FDA issued good laboratory practice regulations on December 22, 1978, which set standards for conducting animal studies.

CFSAN agreed it needed a process to determine the accuracy and reliability of the data in the aspartame studies. This process, known as "authentication," was to determine whether Searle's supporting data from the studies matched its submitted reports to FDA. Authentication would not include reviewing the experimental design of the studies,[4] determining the safety of aspartame, or determining that CFSAN was justified in initially approving aspartame. CFSAN would make the final decision on those issues. Authentication would determine whether CFSAN was justified in using Searle's aspartame studies to support the safety of the compound. To authenticate the studies, FDA chose UAREP, a consortium of nine universities established in 1966.

FDA officials believed the job would be unmanageable if UAREP attempted to review every aspartame study. Therefore, CFSAN used the following criteria for selecting studies:

- Studies ordinarily required by CFSAN to determine safety.
- Studies that, if they had shown a toxic effect, would lead to a different conclusion on safety.
- Studies relating to issues raised by the objectors (see ch. 2).
- Studies selected at random.

By following these criteria, CFSAN selected the following 15 aspartame studies, including 5 of the 11 investigated by the 1975 task force:

- The nine crucial studies (see ch. 2).
- Three studies suggested by the objectors.
- Three random studies.

(See app. II for a list of the 15 studies.)

In April 1977, an FDA team began authenticating 3 of the 15 studies. Later, Searle entered into a contract with UAREP that was agreeable to FDA. This contract stipulated the authentication effort was to be an independent process with neither Searle nor FDA controlling or influencing the work, even though Searle was paying for it. In August 1977, UAREP began investigating the 12 remaining aspartame studies.

[4]Experimental design of the study is the plan for conducting the experiment and is usually written in the protocol that is formulated before the experiment is begun.

FDA Team Investigated Three Searle Studies

An FDA team investigated the Rat DKP Study (one of the crucial studies) and two teratology studies. The investigative team consisted of experienced field investigators supported by CFSAN scientists. In addition, a pathologist from FDA's National Center for Toxicological Research examined 7,872 slides and 7,360 tissue blocks from the Rat DKP Study.

The investigative team identified quality control problems in the three studies investigated. The team submitted its report to CFSAN officials, who concluded the differences between the original and submitted data were not of "such magnitude that they would significantly alter the conclusions of the studies." (See app. IV for a more complete list of problems and CFSAN's comments.) Some of the major problems identified and CFSAN's comments are discussed in the following paragraphs.

1. The investigative team found the diets in the Rat DKP Study may not have been homogeneous because no records existed on tests performed on the feed mixture's composition. Two reports indicated feed samples were not homogeneous. If the feed was not homogeneous, the rats could eat around the DKP and not consume it. Additionally, the team found a photograph of feed in a Searle analyst's notebook that clearly showed DKP particles distributed nonuniformly throughout the mixture.

CFSAN officials could not determine with any certainty that the diets were homogeneous. However, they believed there was evidence that the diets may have been homogeneous because of a dose-related increase in the incidence in uterine polyps and decrease in blood cholesterol levels.

2. In addition, the team found many of the tissues in the Rat DKP Study appeared to have been omitted due to autolysis (a breakdown of all or part of a tissue). The 1975 task force also found evidence of tissue loss from autolysis.

CFSAN officials found the tissue loss from autolysis was distributed among all dose groups and did not appear to occur selectively; e.g., mainly within a particular tissue or group. Hence, they could not determine whether the results would have been altered if these tissues had been obtained before autolysis.

3. According to the investigative team's report, the examination of fetuses and the reporting of the results in the two teratology studies were inadequate. For example, they found that 329 examinations were performed in 2 days—an impossible feat for one person. In addition, not enough tissue sections were taken through the heart.

CFSAN officials noted the Searle scientist who performed these examinations estimated that he examined about 30 fetuses a day, but CFSAN officials could not determine from the available data when these examinations were done. Additionally, instruction manuals were not specific on the number of tissue sections to be taken through the heart. CFSAN concluded

"... while there was no evidence that the study was compromised by this issue, the practice of not making enough tissue sections through the organs, as specified in the protocol, did not preclude a possible failure to observe abnormalities which may have occurred."

In September 1977, CFSAN reported its findings to the FDA Commissioner, who advised Searle that FDA's tentative acceptance of these three studies as authentic reflections of the data in Searle's possession did not constitute an endorsement of the adequacy of aspartame. The Commissioner indicated that the final determination of the scientific merit of these 3 studies, as well as the other 12, could only be made in conjunction with the evaluation of the UAREP report and that FDA would await the completion of the UAREP review before proceeding further.

UAREP Authenticated Remaining 12 Studies

In August 1977, after 8 months of contract discussion between FDA and Searle, UAREP began authenticating 12 studies. According to the UAREP report, it reviewed 2,200 pages of materials submitted by Searle to FDA on the 12 studies. In addition, Searle provided over 21,000 pages of background materials to UAREP. The UAREP pathologists diagnosed 39,000 tissue sections for 4,900 animals, including clinical observations, food consumption, weight changes, clinical laboratory tests, and autopsy results. FDA received UAREP's final report in 1978.

UAREP did not find evidence that animals in any one group had been treated deliberately to produce biased results. They concluded that the data submitted by Searle on the 12 studies were authentic. Although UAREP noted a "substantial number of minor and inconsequential discrepancies" in the studies, "there were few, if any discrepancies which would produce a change of greater than 5 percent in the final numerical data being compared."

One of UAREP's concerns during the authentication of Searle's aspartame studies was to be sure its efforts were free from Searle's influence or even the appearance of influence. Therefore, UAREP documented all communications between Searle and UAREP and eventually turned over these

documents to FDA. According to UAREP's principal investigator, UAREP only provided Searle

"... with drafts of the general introduction, which included formulas for aspartame and how it was metabolized ... Searle did not see a single word of the summary and conclusions until it received a copy of the final report." UAREP told Searle to relay its comments regarding the report to FDA.

At the request of CFSAN, FDA scientists reviewed UAREP's final report to determine whether any discrepancies noted by UAREP were sufficient to invalidate the studies' results. The FDA scientists agreed with UAREP that the data submitted by Searle were authentic. They also commented on the issues noted by UAREP. Some of these issues and the FDA scientists' comments are discussed in the following paragraphs.

1. UAREP noted that during the study, abnormal tissue masses were reported as present and then not observed at subsequent intervals. It believed when multiple pathologists examined tissues, some would miss abnormal tissue masses.

The FDA scientists stated that one would expect to find variations in diagnoses between trained pathologists.

2. Although UAREP noted some differences between its diagnoses and the original ones, UAREP did not believe the differences were significant. The UAREP pathologists reviewed these studies blind.[5] According to UAREP's president at the time of its review,

"... the thing that impressed [UAREP] throughout the study, ... which is reflected in our final statements and conclusions, was that the interpretation of the experimental results by previous observers did not really differ very significantly from ours following our review of the material."

The FDA scientists said the differences between UAREP's diagnoses and the original diagnoses were probably the result of different pathological interpretations. Additionally, one scientist said these differences did not represent an impressive list of discrepancies, considering the large number of microscopic sections involved.

[5]"Blind" means that the pathologist did not know if the tissue slides were from control or treated animals, and was unaware of previous diagnoses. UAREP's principal investigator compared the pathologists' diagnoses with Searle's original diagnoses.

Aspartame Studies Reviewed for Conduct

Searle conducted aspartame studies in the early 1970's, before the implementation of FDA's good laboratory practice regulations. The problems found with Searle's studies resulted in controversy over whether UAREP's and the investigative team's reviews considered how the studies were conducted and whether CFSAN was justified in using Searle's aspartame studies to support its safety.

An FDA scientist stated that a review of the studies' conduct can be done two ways. The first requires that someone be present when the study is conducted to determine exactly what occurs. This, of course, is the most accurate method, but was not practical. In fact, UAREP's principal investigator said it is impractical to have 24-hour surveillance of a study.

The second type of review assesses the conduct of the studies by reconstructing the studies from available supporting data. UAREP scientists and scientists on the investigative team informed us that they reconstructed the studies when they examined the aspartame data. According to UAREP's principal investigator, UAREP looked at the studies' conduct by assessing

- protocols and amendments;
- clinical observations;
- body weight, food, and compound consumption;
- clinical laboratory tests;
- ophthalmoscopic observations;
- necropsy (autopsy);
- survival data;
- histopathology (microscopic examination of the tissues);
- personnel, facilities, and methods;
- animals and animal care; and
- data production, handling, and storage.

For example, UAREP

- reviewed the protocol and amendments to determine whether the experiments were carried out according to the plans;
- examined tissue slides to determine the quality of the preparation of the slides and to verify the diagnoses of the lesions; and
- interviewed some present and former Searle personnel who worked on these studies. In addition, UAREP reviewed curriculum vitae for professional personnel at Searle and Hazleton.

UAREP officials also visited the laboratories that performed the aspartame studies. They noted these laboratories were accredited by the American Association for the Accreditation of Laboratory Animal Care[6] when the aspartame studies were conducted. According to UAREP's principal investigator, UAREP also considered the studies' conduct in reaching its conclusions. UAREP concluded that procedural problems were not of sufficient magnitude to invalidate the studies' conclusions. For example, if some animals' weights were missing, UAREP determined whether the missing weights made any difference in the study's final conclusions. UAREP found the missing weights were "not a major factor." In addition, according to a former president of UAREP, had UAREP "found something that would have affected the study, we would most certainly have reported it."

CFSAN believed that based upon the investigative team and UAREP authentication efforts, it was justified in using Searle studies in 1974 to support aspartame's safety. Based on the authentication efforts, FDA concluded it could hold a public hearing on the objections to aspartame's approval.

Conclusions

FDA investigators and scientists identified problems in a number of the crucial aspartame studies. Some believed the studies could not be used to determine aspartame's toxic potential. In response, FDA had an independent organization and an FDA team investigate the supporting studies for accuracy and reliability and concluded that the studies could be used to assess aspartame's safety. We believe that FDA's actions were appropriate and that UAREP and CFSAN addressed the conduct of the studies.

[6]This association inspected laboratories to determine whether they met certain standards for accreditation.

Appendix II

Aspartame Studies Investigated

The 1975 task force, an FDA team, and UAREP investigated a total of 22 aspartame studies (see ch. 3). The FDA team and UAREP investigated five of the same studies as the task force. However, the FDA team and UAREP investigations were more detailed, identifying problems that had been previously noted by the 1975 task force as well as additional problems.

Table II.1 lists the studies and the group(s) that investigated each study.

Table II.1: Aspartame Studies Investigated

	Studies investigated by:		
Aspartame studies	**1975 task force**	**FDA team**	**UAREP**
Crucial studies:			
Two-Year Rat Study	X		X
Lifetime Rat Study			X
Two-Year Mouse Study			X
Long-Term Dog Study	X		X
Multigeneration Rat Study			X
Supplemental Dog Analysis			X
Supplemental Rat Analysis			X
Rat DKP Study	X	X	
Mouse DKP Study			X
Studies suggested by objectors:			
Newborn Rat Toxicity Study			X
Endocrine Studies			X
Pregnant Monkey Study			X
Other studies:			
Waisman Monkey Study	X		
Hamster Study	X		
Acute Rat, Mouse, and Rabbit Study	X		
Rat Teratology DKP Study Segment I	X		
Rat Teratology DKP Study Segment II	X		
Rabbit Teratology Aspartame Study Segment II	X		
Rabbit Teratology - Phenylalanine/Aspartic Acid Study Segment II	X		X
Mouse Teratology Aspartame Study Segment II	X	X	
Rat Teratology Aspartame Study		X	

Appendix III

Eleven Aspartame Studies Investigated by 1975 Task Force

The 1975 task force investigated 11 aspartame studies (see ch. 3) and uncovered "serious deficiencies in Searle's operations and practices which undermine the basis for reliance on Searle's integrity in conducting high quality animal research to accurately determine or characterize the toxic potential of its products." However, the task force said that unreliability in Searle's animal research did not imply its animal studies provided no useful information on the safety of the products, such as aspartame. They believed the FDA should conclude whether the results from a study could be used in evaluating a product's toxic potential. The remainder of this appendix consists of examples of the 1975 task force findings on the aspartame studies and CFSAN's comments.

Two-Year Rat Study

1975 Task Force Findings

Protocols did not specify the exact procedures for examining certain animals, and certain other experimental procedures were not followed.

The total number of tissues examined included autolyzed tissues, although these tissues should not have been used in calculating the percentage of incidence of certain types of lesions.

An observation from tissue slides was noted in a table of the final report for about 15 tissues. However, no tissue slides were noted as being prepared.

Animals were not tagged to prevent mix-ups.

The presence and the specific concentration of test compounds in the animal feed was not determined.

CFSAN Comments

Investigated by UAREP (see app IV for comments).

Long-Term Dog Study

1975 Task Force Findings

Portions of Searle's submission to FDA were not supported by Searle's records. For example, the submission to FDA stated an evaluation of

behavioral activity was routinely recorded, yet the task force could find no records for this.

Feed containing the test compound was not assessed for homogeneity.

Protocols were not always followed.

The submission states that dogs ranged from 150 to 160 days of age, yet 3 dogs were 220 to 235 days of age. These three dogs were assigned to the treatment groups and none to the control group.

CFSAN Comments

Investigated by UAREP (see app. IV for comments).

Rat DKP Study

1975 Task Force Findings

Control and dosed animals were randomly distributed on the same rack and not tagged to prevent mix-ups.

Feed containing the test compound was not checked for homogeneity, and food spillage by individual animals was not recorded.

Some animals were not autopsied until 1 year after they died.

Portions of Searle's submission to FDA did not include the animals' conditions during the study. For example, animals were infected with a disease during the study and medication was given, but this was not reported in Searle's submission to FDA.

In some cases, protocols were not followed.

CFSAN Comments

Investigated by the FDA team (see app. IV for comments).

Waisman Monkey Study

1975 Task Force Findings

Protocols were written after the study was started and were not followed.

Portions of Searle's submission to FDA were not supported by Searle's records. For example, Searle's submission stated that animals were unavailable for autopsy at the study's termination. However, other documents indicated the animals were available, but Searle chose not to purchase them.

The exact intake of aspartame and DKP could not be calculated from the data submitted.

The Searle scientist listed as the primary author of the study's report was not employed at Searle until 3 months after the study was terminated.

CFSAN Comments

Two years before the task force report, CFSAN noted problems with this study because it was never completed, and the scientist in charge had died. CFSAN considered this study's findings to be of limited value, but indicated the study lent support to the need for labeling (see discussion on this study in ch. 2).

Hamster Study[1]

1975 Task Force Findings

Records of observations on animals were not accurate or consistent. For example, Searle was inconsistent in recording the dates showing when an animal died.

The primary author of the submission to FDA was not employed at Searle until 3 months after the study was terminated.

[1]This study includes three reports submitted to FDA. The 1975 task force investigated and counted the reports as one study.

CFSAN Comments	About 3 years before the 1975 task force report, CFSAN noted this study had been terminated before completion because of an infection in the animals. CFSAN found this study to be of limited value in evaluating the safety of aspartame before the 1975 task force review.

Acute Rat, Mouse, and Rabbit Studies

1975 Task Force Findings	Searle's submission to FDA could not be supported by Searle's records. For example, Searle reported the animals were observed over a 7-day period, which was not supported by data in Searle's records.
CFSAN Comments	CFSAN did not comment on the task force's findings. These studies were not considered to be "crucial studies" for the approval of aspartame.

Five Teratology/ Reproduction Studies

1975 Task Force Findings	The task force concluded that although transcription errors were found in all these studies, the errors would not significantly alter the reported conclusions.
CFSAN Comments	These studies were not considered to be "crucial studies" for the approval of aspartame.

Rat Teratology DKP Study (Segment I)

1975 Task Force Findings	The rats' dosage period was not accurately determined. The actual amount of DKP ingested by the rats could not be determined with certainty.

	On the basis of this study, it was not possible to set a "no adverse effect" level of DKP.
CFSAN Comments	CFSAN did not comment on the task force findings.

Rat Teratology DKP Study (Segment II)

1975 Task Force Findings	In spite of the poor reporting and minor inaccuracies, it was still possible to say DKP at levels as high as 2.4 percent was probably not toxic to the fetuses of the rats.
CFSAN Comments	CFSAN did not comment on the task force findings.

Rabbit Teratology Aspartame Study (Segment II)

1975 Task Force Findings	Conclusions on the effects of aspartame could not be properly assessed due to the poor design and the high animal death rate.
CFSAN Comments	About 3 years before the task force report, CFSAN noted this study precluded a meaningful evaluation of the high dose group for DKP because of the high death rate for animals in this group.

Rabbit Teratology Phenylalanine Aspartic Acid Study (Segment II)

1975 Task Force Findings	Animal groups received the incorrect test compounds throughout the study. However, this study was conceived and conducted at a satisfactory scientific level, and the problems found did not appear to be influential in interpreting the findings of the study.
CFSAN Comments	Reviewed by LARFP.

Mouse Teratology Aspartame Study (Segment II)

1975 Task Force Findings	The study used an inappropriate method of administering aspartame, limiting the conclusions that could be drawn. This study could only conclude daily doses of aspartame from greater than 0 to less than 2 grams would not have toxic effects on the fetus from the 6th to the 15th day of pregnancy.
CFSAN Comments	Reviewed by the FDA team.

Findings of the Reviews on Crucial Studies

In response to the 1975 task force findings, CFSAN selected 15 Searle aspartame studies for review (see ch. 3). UAREP reviewed 12 studies, 8 of which were crucial to aspartame's approval, and an FDA team reviewed 3 studies, 1 of which was crucial. The UAREP and FDA team reports contain many findings, some of which are discussed in this appendix.

UAREP's Comments on Searle's Studies

In carrying out its review of the aspartame studies, UAREP noted that when the Searle studies were performed (1970's), few standards for laboratory work were required. Therefore, UAREP stated it reviewed the studies using methods and interpretation common to research laboratories around 1970.

General Comments on All Studies

UAREP's general comments on issues addressed in the Searle studies follow.

- Animal Care Facilities: The American Association for Accreditation of Laboratory Animal Care had accredited the animal facilities at Searle since 1968 and at Hazleton Laboratories in 1971. UAREP said the fact both Searle and Hazleton had this accreditation while performing the aspartame studies "would indicate that their facilities were far above the average and would be considered quite adequate for that time."
- Personnel, Facilities, and Methods: UAREP stated "there have been many changes in personnel, facilities, and laboratory technology" since the aspartame studies were done. Although the great majority of the staff who carried out these studies were no longer employed by Searle or Hazleton, the scientific personnel with whom UAREP talked during visits to the laboratories exhibited good knowledge of their work and responsibilities.
- Protocols: UAREP found some of the studies' protocols appeared to be more a record of what had been done than a plan of what was to be done. However, UAREP concluded that the scientists knew what they were doing.
- Data Production, Handling, and Storage: UAREP agreed with Searle and Hazleton on more than 99 percent of the computations. They also found "a very small incidence of transcriptional discrepancies."

Although UAREP noted "a substantial number of minor and inconsequential discrepancies" during its review, it found "few, if any, discrepancies which would produce a change of greater than five percent in the final numerical data being compared." In addition, it did not find evidence

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that, "given the experiment design, there was any indication that animals in any one group had been treated deliberately to produce biased results." The discrepancies it observed "appeared randomly distributed between treated and control groups."

CFSAN concluded that, although UAREP found some discrepancies in the aspartame studies reviewed, there were no discrepancies "that were of sufficient magnitude or of a nature that would compromise the data as originally submitted by Searle."

Four Long-Term Rodent Studies

In its report, UAREP grouped some of the eight crucial studies according to the studies' objectives and discussed its findings accordingly. For example, four long-term toxicity studies carried out by Hazleton tested the effects of aspartame or its breakdown product, DKP, in rodents. These studies were the Two-Year Rat Study, the Lifetime Rat Study, the Two-Year Mouse Study, and the Mouse DKP Study. UAREP's findings on these four studies follow.

- Clinical Observations: UAREP was "generally in close agreement" with Hazleton's clinical observations in these four studies.
- Body Weight, Food, and Compound Consumption: Based on the available data, UAREP "did not disagree" with Hazleton's handling of body weight and food consumption data. UAREP said its calculation of consumption at times differed from Hazleton's, but overall, both calculations were in "very close agreement."
- Survival Data: Although UAREP "generally agreed" with Hazleton's report that survival in dose groups was not statistically different, UAREP differed with Hazleton's values for percentage survival at the studies' ends. For example, in one study, this resulted because Hazleton killed animals in the high dose group 2 weeks earlier than animals in other groups and omitted 10 of these survivors in computing the average survival time.
- Clinical Laboratory Tests: These tests had "a scattering of statistically significant differences in various parameters and among various groups." However, UAREP agreed with Hazleton that "under the conditions of these experiments, these differences were neither dose nor compound related."
- Ophthalmoscopic Observations: UAREP noted some of the rats and mice had a clouding of the eye lenses, but found no dose relationship.
- Necropsy (Autopsy): UAREP found that on the basis of the information available, the autopsy records "were reasonably good," and it "generally agreed" with Hazleton's transcriptional and computational data.

UAREP pathologists evaluated the issue of autolysis noted by the 1975 task force. They found autolysis was randomly distributed among the studies' animals; therefore, they did not consider this to significantly affect the studies' results.

- Histopathology: UAREP pathologists examined 35,000 tissue sections without knowing the original diagnoses made by Hazleton's subcontractor. UAREP stated a "good correlation" existed between its diagnoses and those of the subcontractor. In addition, UAREP believed the differences noted and an occasional missing slide, "did not significantly affect" the results' interpretation. UAREP commented on the higher tumor incidence found in the controls and in the low dose groups, and said "there was no evidence that either aspartame or DKP enhanced the production of tumors in these studies."

Long-Term Dog Study

UAREP examined Searle's Long-Term Dog Study and commented on the following:

- Clinical Observations: UAREP was unable to evaluate the adequacy of clinical observation procedures in this study because it was not supplied with any records of clinical observation data.
- Body Weight, Food, and Compound Consumption: UAREP found the amount of aspartame consumed was "somewhat variable, but never was more than 6 percent from the desired dose." In addition, it said "the randomization of animals was done haphazardly" because two or three dogs from the same litter were in the same group, and the dogs with significantly higher body weights were in the high dose groups at the study's beginning.
- Clinical Laboratory Tests: UAREP noted "fewer significant differences" on blood tests between the dose and control groups than reported by Searle. However, Searle's data showed a significant number of red cells in the urine of some dogs. UAREP believed "these red cells would have produced bloody urine or resulted from urinary tract disease, but Searle's records did not report observing either bloody urine or urinary tract disease." Therefore, UAREP questioned the validity of these data.
- Ophthalmoscopic Observations: UAREP found two animals with cataracts, but determined these cataracts to be congenital.
- Necropsy (Autopsy): UAREP said the quality of tissue sections was generally good at the time of its review. In addition, UAREP found no discrepancies in Searle's transcription of organ weights from autopsy sheets to the report to CFSAN.

- Histopathology: UAREP's review of tissue slides showed "only two significant discrepancies" in the diagnoses when compared to Searle's diagnoses.

Supplemental Dog Analysis and Supplemental Rat Analysis

Because of a possible increased incidence of brain tumors in the Long-Term Dog Study, the Two-Year Rat Study, and the Lifetime Rat Study, Searle hired a pathologist to review additional brain tissue sections. The Supplemental Dog Analysis and the Supplemental Rat Analysis contained the results of the pathologist's review. UAREP convened a panel of experts to review the pathologist's findings on these studies. UAREP "agreed completely" with Searle's pathologist that there were no brain tumors in the dog brain tissue sections examined, nor other significant pathologic lesions relating to aspartame. In addition, they "generally agreed" with Searle's pathologist on the diagnoses of the rat brain slides and said "the 20 brain tumors diagnosed showed no statistically significant increase in any group when the tumors for the [Two-Year Rat Study and the Lifetime Rat Study] were combined."

Multigeneration Rat Study

Hazleton Laboratory performed the Multigeneration Rat Study reviewed by UAREP. UAREP noted "the consumption of aspartame was from 25 to 38 percent lower than planned at certain stages of the study." However, UAREP found "fewer discrepancies or problems in this study than in most of the other studies [it] reviewed."

FDA Team Comments on the Rat DKP Study

The FDA team examined one crucial study, the Rat DKP Study, and submitted its findings to CFSAN, which concluded that the concerns identified would not significantly alter the study's conclusions. This study was to determine DKP's safety and its potential to produce tumors. Some of the problems identified by the investigative team and CFSAN's comments are shown in table IV.1.

Table IV.1: FDA Investigative Team Concerns and CFSAN's Comments

Concerns identified by the FDA team	CFSAN comments
Eleven transcriptional errors were noted when comparing the organ weights from the original data with those weights submitted to FDA.	CFSAN used the corrected values in recalculating these data and found "the differences did not appear to significantly alter the submitted data."
The blood and urine data revealed 21 differences between Searle's submitted values and those in Searle's original data.	CFSAN recalculated these data and found they "did not differ statistically" from the results Searle reported to FDA.
Searle did not report a third outbreak of an infectious disease to FDA.	CFSAN said this outbreak should have been reported. However, this disease only involved four animals, and records from the study showed no increase in the death rate of any group because of this infection. All surviving animals received treatment. These unreported facts "would not by themselves appear to affect the interpretation of this study."
The values to determine blood cholesterol were not reported for two days but appeared in Searle's records.	CFSAN calculated the unreported values and found they did not differ "significantly from the values reported for the other days."
Values to determine blood chemistries were not reported for two days, but appeared in Searle's records.	CFSAN said "although these values were not included in the submission to FDA, the omission would not appear to affect the results, since the findings are similar to those for the reported days."
In three instances, Searle's submission to FDA showed a pathology diagnosis for certain organs. However, Searle's records indicated these organs were missing.	CFSAN said the inclusion of any of the three diagnoses would not alter the conclusions.
Records of approximately 30 animals showed differences between the original pathology sheets and Searle's pathology summaries submitted to FDA. For example, several observations were omitted in the submitted data.	Although the omitted lesions should have been reported, CFSAN believed some of these lesions "could have been considered insignificant by some pathologists." CFSAN said the FDA investigative team pathologist's review of 20 percent of the tissue slides generally "showed agreement" between his findings and Searle's.
The protocol was not always followed. In many cases, the actual number of organs prepared was less than what was specified in the protocol.	CFSAN's review of Searle's data indicated many of the organs appeared to have been omitted due to autolysis. They said this loss was distributed among all groups and did not appear to be selective to particular organs or groups. CFSAN could not determine whether the results from this study "would have been altered" if these organs had been examined before autolysis.
Animals were not individually labeled; only the cages were labeled. In addition, the chances of administering the wrong diet to the animals was greatly increased by using unlabeled feeding jars.	CFSAN could find no evidence to suggest any feeding errors occurred. They could not determine whether any dietary mix-up occurred, because no feeding procedures existed for this study. However, they decided the increased incidence of uterine polyps and the decreased levels of blood cholesterol suggested mix-ups may not have occurred and the rats ate the DKP.

Concerns identified by the FDA team	CFSAN comments
A photograph was found in a Searle analyst's notebook which showed "discrete light-colored particles of varying sizes and shapes distributed nonuniformly throughout the feed." These particles were DKP. According to Searle's records, these samples were not homogeneous and had to be reground before sampling. The FDA team found no evidence these diets were reground before being fed to the rats.	CFSAN could not determine from the available information whether the diet was homogeneous and could not determine the actual amount of DKP consumed. Additionally, CFSAN stated the FDA investigative team could find no documentation on how the feed was prepared or whether these samples were representative of the rats' feed.
Differences in animal data were found between Searle's records and its submission to FDA.	CFSAN said these differences did "not appear to alter the interpretation of this study."
A tissue mass was removed from a high dose animal. In addition, incisions were made over tissue masses on two low dose animals, and the animals were continued in the study.	CFSAN said even though the removal of the mass from the high dose animal was reported to the FDA, "such an early excision can prevent the progression" to a malignant tumor. The masses on the two low dose animals appeared one week after the animal housing area was sprayed with a rodenticide, but these masses disappeared during the study.
At times, Searle changed the clinical laboratory procedures during the study	CFSAN said Searle's submission should have reflected these changes in the procedures. Although it was "not unusual" to change a procedure during a study, CFSAN noted such a change could "conceivably result in differences" in the data. However, they concluded Searle's changes would "not appear to invalidate" this study.