

AUTHENTICATION REVIEW OF SELECTED MATERIALS SUBMITTED TO
THE FOOD AND DRUG ADMINISTRATION RELATIVE TO APPLICATION
OF SEARLE LABORATORIES TO MARKET ASPARTAME

Volume No. 2

Chapter IV: Two Year Toxicity Study in the Rat
Chapter V: Lifetime Toxicity Study in the Rat
Chapter VI: 104 Week Toxicity Study in the Mouse

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UNIVERSITIES ASSOCIATED
FOR RESEARCH AND EDUCATION IN PATHOLOGY, INC.

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SUBMITTED TO THE FOOD AND DRUG ADMINISTRATION
RELATIVE TO APPLICATION OF SEARLE LABORATORIES TO MARKET ASPARTAME

prepared by
Universities Associated for Research & Education in Pathology, Inc.

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CHAPTER IV

E-33,34: ASPARTAME: TWO YEAR TOXICITY STUDY IN THE RAT

INTRODUCTION

Most materials submitted by Searle to FDA as an Entry consist of two parts: a summary of the experiment with results and conclusions and an appendix of detailed tabular data. In this instance, separate numbers were given to these two parts, with E-33 being the Appendix and E-34 being the general presentation. In our write-up, when figures, tables, or page numbers are cited, we will indicate either E-33 or E-34, and when making reference to the entire study, E-33,34 will be used.

Searle Laboratories contracted with Hazleton Laboratories, Inc., now known as Hazleton Laboratories America (HLA), to conduct this two year oral toxicity study to evaluate aspartame in the rat (P-T No. 838H71; FDA Entry Book E-33,34). This was accomplished by feeding aspartame at four dosage levels to Charles River cesarean-derived (CRcd) strain of rats for a period of 104 weeks during which time clinical observations, body weight changes, food and compound consumption, ophthalmoscopic observations and various hematologic, clinical and special chemistries, urinalysis, histopathologic, necropsy and other observations were made on the animals. The experiments began December 15, 1969 and ended December 14, 1971.

Personnel

The following personnel from Searle Laboratories were members of the Searle protocol design committee for E-33,34:

Dr. Sammeta.....Biostatistician

Dr. F. Saunders.....Biology Research Advisor

Dr. Ranney.....Metabolism Representative

Dr. Polk.....Clinical Representative

Dr. McConnell.....Pathology-Toxicology Department Advisor

Dr. Hutsell.....Bioanalytical Laboratory

Dr. Rao of Searle Laboratories, Inc., acted as the liaison between Searle Laboratories and Hazleton Laboratories for the experiment E-33,34.

For Hazleton Laboratories, Dr. D. W. Jessup was Project Coordinator and Dr. Frederick E. Renc was Project Manager. The histopathology diagnoses were made by Drs. John F. Ferrell and William M. Busey of Experimental Pathology Laboratories, Herndon, Virginia.

General Experimental Design

Hazleton Laboratories received their protocol from Searle dated October 9, 1969, as the experimental outline for E-33,34 (Hazleton Project Number 700-233). The specifications of this Searle protocol are given in Appendix IV-1.

Clinical Laboratory Studies

Five male and five female animals from the control and each treatment group were to be evaluated at the 6, 13, 26, 52, and 104 week

intervals for hematology (hematocrit, hemoglobin, erythrocyte count, total leukocyte count, differential leukocyte count). The following clinical laboratory determinations were to be made on the same five individual animals at the same intervals: fasting blood sugar, blood urea nitrogen, total serum protein, total serum bilirubin, serum glutamic-pyruvic transaminase, serum alkaline phosphatase, and serum electrophoresis. In addition to the above clinical laboratory determinations, the following determinations were to be made on blood serum at the termination of the experiment at 104 weeks: albumin, sodium, potassium, carbon dioxide, calcium, chloride, and glutamic oxaloacetic transaminase.

Urinalyses were performed on individual animals based on a sample size of five animals, at the following intervals: 6, 13, 26, 52, and 104 week intervals. The following parameters were to be evaluated: pH, specific gravity, glucose, ketones, total protein, bilirubin, and microscopic examination of sediment. In addition to the above determinations, phenylketonuria was to be monitored on a monthly basis using a dipstick method.

Animal and Experiment Conditions

At the initiation of the experiment, the CRcd rats (males 75-108 grams; females 80-102 grams) were individually housed in elevated wire mesh cages. Purina Laboratory Chow and water were provided ad libitum to the animals throughout the experiment. The animals were assigned to various groups by a stratified by weight randomization procedure to

obtain a homogeneous distribution of animals for each experimental group. The aspartame was mixed with Purina Laboratory Chow meal in a Patterson-Kelley twin shell blender. The following animal groups were employed:

1. Control, with no added aspartame
2. Low dosage (1 g/kg body weight/day)
3. Medium dosage (2g/kg body weight/day)
4. High dosage (4 g/kg body weight/day)
5. Very high dosage (6-8 g/kg body weight/day)

This very high dosage group received 6 g/kg of body weight for the first 16 weeks, then 7 g/kg of body weight per day to week 44, and then 8 g/kg of body weight per day for the remainder of the 104 week experimental period. There were 60 male and 60 female rats in the control group and 40 male and 40 female rats in each of the four groups receiving aspartame. The concentration of aspartame in the various diets was adjusted based on the previous week's mean feed consumption, and body weight for the respective groups.

Experimental Animals

Weanling albino CRcd rats (270 males and 270 females) were received by Hazleton December 9, 1969, which was six days prior to the initiation of the experiment. They ranged in weight from 39 to 67 grams for the males and 43 to 64 grams for the females. As mentioned in Chapter II, Hazleton said that the animals were held in isolation for 14 days, which was subsequently restated to be one week, prior to being placed on experiment. On initial examination, 16 male and 19 female rats were

eliminated for abnormalities, primarily eye lesions. The rats used at the start of the experiment ranged from 75 to 108 grams for the males and 80 to 102 grams for the females. At the initiation of the experiment, three additional males and seven females were removed from the study because of abnormalities. The mean initial starting weight for the male rats was 94 grams and for the females, 89 grams.

Protocol and Amendments

According to the terms of the UAREP-Searle contract, under II, Scope of Work, D-1, "UAREP shall review the protocol and any amendments thereto, and reports as submitted to the FDA (hereinafter 'submitted reports') in order to become familiar with the study's objectives and methodology employed." (Appendix II-2). The initial protocols were generally not available to UAREP from either Searle or Hazleton. Recipients of revised protocols were requested to destroy all earlier copies. On the basis of the written materials provided UAREP, it was not feasible to reconstruct precisely the changes which occurred in the original protocol which formed the basis for the study background (Appendix IV-2). Sheets of information on which Appendix IV-2 is based are included in Appendix IV-1.

Only one of the seven Hazleton project sheets was found to be supported by written Searle amendments. Only one of the two Searle amendments provided UAREP by Hazleton and Searle was reflected on a Hazleton Project Sheet. Based on information provided UAREP by Searle

and Hazleton, on only one occasion did a Searle amendment document a Hazleton Project Sheet. Dr. Fred Reno, Hazleton Project Director, did volunteer the information that he and Dr. McConnell, the head of Searle Pathology-Toxicology, frequently discussed their experiments by telephone.

Statistical Evaluation of Data: The Hazleton Project Sheet No. 1 dated December 4, 1969, only indicated the parameters which would receive statistical evaluation without any details as to the methods of evaluation. The Searle protocol stated on page 4, that body weight change; food and drug consumption; and all clinical laboratory values, should have group means \pm standard error, with appropriate analysis of intergroup variance at each time interval. For the mean incidence onset of neoplasms, there should be appropriate analysis of intergroup variance at termination using a life table method. When Dr. Reno of Hazleton was queried as to how methods of statistical analysis were determined, he stated that they always applied the methods which were specified by the sponsor of their work (Searle). The write-up in E-34 prepared by Hazleton states on page 14, "Methods Employed: Life-Table technique for survival; t-test for clinical laboratory data; analysis of variance, or F-test at <5.0% probability level for the remaining criteria; preliminary tests (where applicable) by methods of Bartlett, Rao, Scheffe, Sachs, and Fisher-Behrens (modified t-test); and Life-Table technique for probability of tumor formation."

E-33,34 and E-70 provide identical information regarding statistical methods to be employed. When one studies the results as reported

in E-33,34 as well as in E-70, the only information provided is whether a significant difference was noted. Details as to the specific test or tests employed, degrees of freedom, etc., are not clearly stated. Although it appears that the life table method of Sachs was used for determining survival and probability of tumor formation, and that the t-test was used for clinical laboratory data, it is not clear specifically when or what use was made of "analysis of variance, or F-test, at the $< 5.0\%$ probability level for the remaining criteria; preliminary tests (where applicable) by the methods of Bartlett, Rao, Scheffe, Sachs, and Fisher-Behrens (modified t-test)." Because of the vague and somewhat imprecise information provided in the protocol and Entry Reports for E-34, E-70, E-75, and E-76, UAREP was not always certain that they were applying the precise statistical methods employed by Hazleton. We attempted to do this, but when we could not be certain, we used methods that would generally be regarded as appropriate and applicable. For comparative purposes, we sometimes did this in addition to applying the methods used by Hazleton. Other aspects of UAREP's approach to statistical analyses in these reports is presented in Chapter II.

RESULTS AND DISCUSSION

Clinical Observations

Motor and behavioral activities were observed daily and summarized and recorded weekly. No other neurological evaluations were specified. Palpation for tissue masses or nodules of rats was requested in the initial Searle and Hazleton protocols to be performed at the same intervals as the determinations of body weight and food consumption. Although the rats were examined for any visible or palpable abnormalities, UAREP concerned itself principally with the findings which might possibly be related to tumors. A summary of palpable tissue masses and nodules recorded by HLA and UAREP is given in Table 4-1. Entry Book E-34, p 43, summarized the incidence of palpable tissue masses and nodules

Table 4-1

Number of Rats with Palpable Nodules, Tissue Masses,
or Wart-like Lesions

<u>Group</u>	<u>Males</u>		<u>Females</u>	
	<u>UAREP</u>	<u>HLA</u>	<u>UAREP</u>	<u>HLA</u>
1	4/60	5/60	35/60	36/60
2	13/40	7/40	23/40	22/40
3	5/40	3/40	21/40	20/40
4	5/40	4/40	25/40	25/40
5	7/40	8/40	19/40	20/40

for the various groups. UAREP and HLA agreed on three of the ten groups. The significantly higher incidence of palpable nodules and tissue masses in the female rats is thought to relate to the superficial location of their mammary tumors.

During the process of UAREP's validation of E-33,34, the variations in clinical observation relating to the consistency of reporting nodules and tissue masses were determined and are summarized in Appendix IV-3. On 38 occasions, tissue masses or nodules were reported as present at one of the designated observation intervals and then not observed at subsequent intervals.

UAREP does not wish to attempt to interpret these variations in recorded nodules and tissue masses. Considering the large number of such clinical observations made, the 38 instances is not surprising. Those experienced in recording their own observations on lumps and bumps in experimental animals note that on occasion, areas of active mammary tissue, lymph nodes, focal inflammation or focal fluid accumulation, may appear to grow or to subside and disappear. This is especially true of small, scarcely discernable lesions, when examined sequentially by multiple observers. It is not feasible to ascertain the extent to which the variations in the Hazleton clinical observations were natural phenomena as opposed to errors of observation or recording.

Body Weight Changes

The revised Hazleton Project Sheet specified that body weights should be determined at the following intervals:

"Diet and Compound Administration - In order to ensure proper dosing on a g/kg body weight basis during the weeks of rapid growth, the following regimen will be followed:

- Weeks 0 through 4 - Calculate individual food consumption three times per week, and adjust dose according to body weight change.
- Weeks 5 through 13 - Calculate individual food consumption two times per week, and adjust dose according to body weight change.
- Weeks 14 through 26 - Calculate individual food consumption every week, and adjust dose according to body weight change.
- Weeks 27 through 52 - Calculate individual food consumption biweekly, and adjust dose according to body weight change.
- Weeks 53 to termination - Calculate food consumption monthly and adjust dose according to body weight change."

This schedule was followed with minor modifications shown in Appendix IV-4. Subsequent Searle protocol amendment dated October 7, 1971 (approximately 22 months after initiation of experiment) specified that body weights were to be determined weekly. Body weights were determined as outlined in the Hazleton Project Sheet dated December 4, 1969, and

summaries based on those interval frequencies were calculated in Entry Book E-33, Appendix Table No. 1 (pp 3-10).

UAREP body weight computation agreed with the 500 means reported in Appendix Table No. 1 of Entry Book E-33. A summary of the statistically significant differences between groups at various intervals for males and females is given in Appendix IV-5. Hazleton only compared weight gains of treatment groups to controls for the first year, without specific mention of statistical analysis; UAREP compared all groups with each other with statistical analysis for the full two years. UAREP found that female Groups 1, 2, and 3 weighed significantly more than Group 5 for weeks 12 to 84 and that Group 4 weighed more than Group 5 females for weeks 26 to 84. Group 5 males weighed less than the other four treatment groups from weeks 26 to 104. These findings are in agreement with the more general expressions of weight changes for the first year made by Hazleton.

Food Consumption

The rats received Purina Laboratory Chow and water ad libitum throughout the experimental period. Food consumption was determined at the same intervals as body weight and clinical observations. Special notebooks were maintained on an individual animal basis for 0 through 68 weeks, after which time food consumption was entered into the INTEC computer system which was used for processing and storing body weights, food consumption, and clinical observation data.

A summary of food consumption for the intervals 0 through 26, 0 through 52, and 0 through weeks 100 is contained in Table 4-2. During the last year of the experiment, food consumption was recorded monthly

Table 4-2

Summary of Food Consumption (g) For 0 to 26,
0 to 52, and 0 to 100 Weeks

Group	Weeks 0-26			Weeks 0-52			Weeks 0-104		
	\bar{x}		SD	\bar{x}		SD	\bar{x}		SD
1M	4215	±	310	6032	±	496	7980	±	591
2M	3992	±	398	5891	±	540	7766	±	590
3M	4179	±	384	6203	±	395	8041	±	490
4M	4041	±	255	5878	±	386	7706	±	502
5M	3918	±	238	5668	±	344	7434	±	433
1F	3163	±	251	4690	±	380	6254	±	547
2F	3139	±	220	4678	±	379	6228	±	625
3F	3176	±	277	4632	±	374	6172	±	602
4F	2990	±	215	4426	±	372	5628	±	420
5F	2935	±	190	4281	±	318	*	±	--

* animals sacrificed at 102 weeks instead of after 104 weeks

in the earliest data source available to UAREP which was the individual animal food consumption and body weight and clinical observation records. This raised the question whether new food was provided only once monthly. During a visit by UAREP representatives, Hazleton personnel indicated that the feed was changed every week (See Appendix II-9). Subsequently, Hazleton indicated that only the food consumption for the last week of the interval was recorded and all previously uneaten diet in feeders was discarded without being weighed.

Some would feel that it would be more accurate to determine food consumption over more than the last fourth of the feeding period. Others would feel that this would give a reasonable approximation since food and compound consumption was based on the mean consumption of groups, which, even late in the experiments, were of moderate size, and that four different treatment levels would offer opportunity to test a dose response. For reasons discussed later, the measurement of food consumption by change in weight of the feeder is not necessarily a highly precise indication of food assimilation by the rat. The stability of aspartame in the diet was not discussed in the Entry Book E-33,34 and is beyond the scope of UAREP's validation of the Entry Book as mentioned in Chapter II.

HLA reported (E-34, p 18) that food consumption over the first year showed a dose related decrease in male Groups 4 and 5 and female Groups 3, 4, and 5 and that this was statistically significantly lower for male Group 5 and female Groups 4 and 5. A dose related trend continued during the second year for Group 3 females and for both sexes in Groups

4 and 5. They state that food consumption was not evaluated, (presumably statistically), after week 52 and do not indicate what methods of statistical analysis were used during the first year.

UAREP checked mean food consumption throughout the experiment and all values agreed with Hazleton's. By checking each mean value against not just the controls, but all other groups simultaneously with Analysis of Variance, we demonstrated the presence of significant differences ($P < 0.05$) which were delineated by the Newman-Keuls and Least Significant Difference (Table 4-3). Males in Group 5 had significantly decreased food consumption as compared with Groups 3 and 1 at 0 to 26, and 0 to 100 weeks and Groups 4, 3, 2, and 1 at 0 to 52 weeks. Females in Group 5 had significantly decreased food consumption as compared with Groups 3, 2, and 1 and 0 to 26, 0 to 52, and 0 to 100 weeks and Group 4 at 0 to 52 weeks. Females in Group 4 had decreased consumption as compared with Groups 3, 2, and 1 at 0 to 26, 0 to 52, and 0 to 100 weeks. Males in Group 4 had decreased food consumption as compared with Group 3 at 0 to 52 weeks and 0 to 100 weeks. UAREP results thus reinforce the significance of Searle's statement of dose-response decreased food consumption, particularly in females, but also in males.

Compound Consumption

The administration of a prescribed amount of compound per unit body weight to rodents can be a complex problem. The method employed in this study required adjusting the concentration of aspartame in the food on the basis of the previously determined mean body weight and consumption

Table 4-3

Statistically Significant Differences Between Groups for Food Consumption at 0 to 26, 0 to 52, and 0 to 100 Weeks by Analysis of Variance (ANOVA), Least Significant Difference, and Newman-Keuls all at $p < 0.05$

Rat Group	Week Interval → 0 to 26		0 to 52		0 to 100	
	Sex → M F		M F		M F	
	ANOVA →	.00 .00	.00 .00	.00 .00	.00 .00	.00 .00
5<4		∅ ∅	+ ∅	∅ ∅	∅ ∅	∅ ∅
5<3		+ +	+ +	+ +	+ +	+ +
5<2		∅ +	± +	+ ∅	∅ +	+ +
5<1		+ +	+ +	+ +	+ +	+ +
4<3		∅ +	+ +	+ +	+ +	+ +
4<2		∅ +	∅ +	+ ∅	∅ +	+ +
4<1		∅ +	∅ +	+ +	+ +	+ +
3>2		± ∅	+ ∅	∅ +	+ ∅	∅ ∅
3 vs 1		∅ ∅	∅ ∅	∅ ∅	∅ ∅	∅ ∅
2 vs 1		∅ ∅	∅ ∅	∅ ∅	∅ ∅	∅ ∅

+ indicates a significant increase in food consumption by all three methods of statistical analysis; ± indicates a significant increase by Least Significant Difference, but not by Newman-Keuls; and ∅ indicates that neither the Newman-Keuls nor Least Significant Difference are significant at the $p < 0.05$ level.

of diet (based on weight changes in the animal's food container plus food). It is impractical and probably unnecessary to attempt this on an individual rat basis, when one can use mean determinations of a group to average out the individual fluctuations in body weight and food consumption. Fortunately, the Hazleton rats were fed with "improved" feeders and their rats were better trained than those used by some UAREP scientists who have encountered problems correcting for the amount of weight added to the feeders by the rodents' feces and urine and the amounts of food removed from the feeder but not eaten by the rodent. Nevertheless, one might hope to achieve mean assimilation over a long term experiment within approximately 10-20% of the expected amount, which with more than a 6-fold range of dosage as Searle employed, would provide relative dose-response testing situations.

Since it would be cumbersome to reproduce the graphs UAREP prepared, its analysis of the fluctuations in the calculated amount of the compound consumed (removed from the feeder) as compared with the compound consumption prescribed in the protocol, is shown in Table 4-4. If one disregards the 10 days' time during the two year experiment when all experimental animals received control diet, the rats received between 90 and 100% of the expected amounts of aspartame at 80% of the diet administration intervals. A Hazleton internal memorandum from Paul Upman to "Mixing Records" indicated that because of a computational error the test animals received about 1/3 of the desired dosage for days 3 and 4 of the experiment.

Survival

Hazleton Project Sheet No. 1 & 2 dated December 4, 1969 indicated

Table 4-4

Deviations From Planned Compound Consumption
for both Male and Female Rats in Various Groups Throughout Experiment

Rat Group	Planned Dosage	Deviation from Plan		Percent Intervals	
		Low	High	Under 90%	Over 110%
2	1 gm/kg	56%	126%	11%	5%
3	2 gm/kg	35%	122%	13%	2%
4	4 gm/kg	37%	112%	16%	3%
5	6,7,8 gm/kg	33%	138%	20%	14%
	6 gm/kg	33%	139%	27%	5%
	7 gm/kg	87%	111%	12%	3%
	8 gm/kg	87%	116%	6%	15%

The above computation of minimum compound consumption does not recognize the time intervals totalling 10 days between the 14th and 20th weeks when all rats were given control diet because they ran out of aspartame.

that survival tables would be furnished Searle in the final report on survival data. No specifications concerning the exact type of survival data or method of analyzing survival data were indicated on this project sheet. Searle's protocol dated October 7, 1971 gave no specifications with respect to how survival data were to be collected, summarized, or presented in the final report. A "life-table technic for survival," was used for survival analysis and E-34, p 23 states that Group 5 females' survival was significantly less than controls.

Statistical Analysis of Survival: The protocol and experiment design called for HLA to apply a life table analysis technique to their survival data. The E-34 report of results does not clearly indicate the method of statistical analysis employed by HLA. They do state that the only statistically significant difference they found was in the very high dose female rats as compared with the controls. The precise data which HLA used in their analysis is not evident to UAREP.

UAREP recomputed mean survival summary information for each of the groups based on death dates reported in clinical observations, summary sheets, and on the individual animal necropsy sheets. The results are recorded in Table 4-5. Mean survival time in days as reported in the Entry Book for Group 5 females, is 423 days. UAREP's computation shows that the Group 5 female mean survival time would be 602 days, which differs significantly from that reported in the Entry Book. Apparently, the main reasons for this decrease were their sacrifice two weeks before all other rats and the failure of HLA to include the ten rats that lived

Table 4-5

Comparison of UAREP and HLA Data (E-34, page 23) for Mean
Percent Survival \pm Standard Error and Mean Survival
Time in Days

A. Percent Survival \pm Standard Error at 104 Weeks

Group	Males		Females	
	HLA % S.E.	UAREP % S.E.	HLA % S.E.	UAREP % S.E.
1	38.4 \pm 6.3	38.3 \pm 6.3	46.7 \pm 6.5	46.7 \pm 6.4
2	45.0 \pm 7.9	45.0 \pm 7.9	57.5 \pm 7.9	57.5 \pm 7.8
3	52.5 \pm 7.9	52.5 \pm 7.9	50.0 \pm 8.0	50.0 \pm 7.9
4	57.5 \pm 7.9	57.5 \pm 7.8	35.0 \pm 7.6	35.0 \pm 7.5
5	52.5 \pm 7.9	52.5 \pm 7.9	25.0 \pm 6.9	25.0 \pm 6.8
Life Table	N	1<5(P=.04)	S ⁻ ND ND	1>5(P=.02) 2>5(P=.01) 3>5(P=.04)

B. Mean Survival Time (Days)

Group	HLA	UAREP	HLA	UAREP
1	569	581	657	659
2	629	630	663	664
3	636	640	640	650
4	666	668	613	614
5	651	661	423	602
ANOVA	ND	.02	ND	.03
Newman-Keuls	ND	NS	ND	NS
LSD	ND	1<5	ND	1>5
	ND	1<4	ND	2>5

ND = Not Done

N = Not significant at P<0.05

to 102 weeks in computing their mean survival.

Based on UAREP's statistical analysis, comparisons between groups as determined by the Analysis of Variance for the male and female groups, were both above the $P=.05$ level so other tests were not done. Mean survival data indicate that the male groups' survival time was increased with the administration of aspartame while that of the female groups' was reduced.

Using a life table method of analysis as outlined in Chapter II, UAREP compared the survival data of all the groups of the same sex. The figures for percentage survival rate derived from the life table analysis were essentially identical for HLA and UAREP as shown in Table 4-5. In male groups, Group 4 had a better survival than Group 1 by a probability of 0.04. Among female rat groups, Group 5 had a higher mortality rate than Group 1 ($P=.03$) and Group 2 ($P=.02$). Thus, UAREP found in the males, the animals receiving 4 gm/kg/day aspartame appeared to survive longer than the rats receiving none, whereas in the females, the survival rate among the rats receiving 6-8 gm/kg/day of aspartame was lower than those receiving none, or 1 gm/kg/day of aspartame. HLA reported that Group 5 females had significantly lower survival than controls at $P < 0.05$. Thus, survival data suggests that male survival is increased with administration of aspartame while the survival of the females is reduced. No explanation of the difference in survival between two sexes in rats receiving aspartame was offered in the Entry Book. UAREP is specifically prohibited by its contract from making any inferences relevant to the effects of aspartame on longevity in man and believes that the results in the rat are spurious, rather than compound related.

Clinical Laboratory Studies

Hematology - Hazleton Project Sheet No. 1 and 2, dated December 4, 1969, and Searle Protocol Amendment No. 2 both specified that hematocrit, hemoglobin, erythrocyte count, total leucocyte count, and differential leucocyte count were to be determined at the 13, 26, 52, and 104 week intervals on five animals from each of the treatment groups. Entry Book E-33, Appendix Table No. 3, summarized the results of these hematological findings. An HLA internal memorandum dated March 23, 1970 stated that animals would not be sampled for the thirteenth week since they would not have received compound since March 20, 1970.

Appendix IV-6 lists the animals that were used as the source of blood for each of the intervals. The first five animals in numerical order from each group were sampled at each of the subsequent intervals until the animal was removed from the experiment (death, accidental death, etc.) at which time the animal was replaced with the next animal in numerical order from that group. For example, only three, two, three, one, and one of the initial five male rats bled for hematology specimens in the Groups 1, 2, 3, 4, and 5 respectively were sampled at the terminal interval.

Discrepancies noted by UAREP in the hematology data contained in Entry Book 33, Appendix Table No. 2 (pp 16-31) are summarized in Appendix IV-7. As compared with the earliest data source, there were no transcriptional errors noted in the 800 entries comprising the hematology data contained in the E-33 Appendix Table 2. Of the 320 means and standard deviations computed and recorded in the final report, ten inconsequential rounding discrepancies were noted which would not change

the interpretation of the data. Appendix Table No. 2 of the Entry Book E-33 reported nine statistical significant differences between treatment groups and controls for the four parameters (Hgb, Hct, RBC, WBC) monitored at the four intervals. Each of these nine significant t-tests were confirmed by UAREP. However, in three of the nine instances, the Analysis of Variance was found not to be significant at the 5% level of probability when performed by the UAREP validation team. In addition to the three discrepancies noted with respect to significant statistical differences between groups, an additional eight significant statistical interactions between treatment groups were noted in the hematology data and are given in Appendix IV-8. These eight comparisons were not reported in the Entry Book because HLA did not evaluate statistical interactions between treatment groups. UAREP prepared graphs of the various hematological parameters (Hct, Hgb, RBC, WBC) and these are shown in Figure 4-1.

Appendix IV-9 shows the confidence intervals for the hematology data. Based on the variance found within the control groups, these indicate the range outside of which values would be required to fall in order to be statistically significantly different from the controls for each of the intervals evaluated. The computation of the confidence interval is based on the equation:

$$\bar{X} \pm t_{0.05} \sqrt{\frac{(\bar{X} - \bar{X})^2}{n - 1}} / \sqrt{n}$$

\bar{X} = mean

$t_{0.05}$ = value from t table based on degrees of freedom

X = observed value

n = number of observations

In order for a statistical significant difference, ($P < 0.05$) to be determined, the mean of treatment group would need to exceed the lower or upper limit of the confidence level.

Blood Chemistry Determinations - Hazleton Project Sheets No. 1 and 2 dated December 6, 1969 and Searle Protocol dated October 6, 1971 (Appendix IV-1) agreed with respect to which clinical chemistry parameters were specified at what intervals, with two exceptions. These will be discussed under urinalysis and phenylalanine determinations.

Blood was collected from the tail vein at the 13, 26, and 52 week intervals; and by arterial puncture at the termination of the experiment. For the 13th and 26th week intervals, the same rats were bled from each group. The animals used for the collection of blood samples for clinical chemistries were usually not the same as those used for the collection of samples for hematology determinations. They are listed in Appendix IV-6A, B. In a number of instances, rats were used as blood sources for clinical chemistries for a number of intervals and then used for blood sources for hematological determinations. The exact criteria for determining which animals were to be used for both hematology and clinical chemistries are not discussed in the Entry Book. Table 4-6 lists the animals and the intervals for which they were used as the blood source for both the clinical chemistries and the hematology. Of the five groups of five male and five female rats (50 total) initially used for chemistries at 13 weeks, none of the same rats were represented as the source of specimens collected terminally at 104 weeks. For the control male rats, the five specimens drawn at the four time intervals (20 total specimens) came from 14 different rats (Appendix IV-6A, 6B).

Table 4-6

Intervals at Which Rats Were Bled for Both Hematology and
Clinical Chemistry Samples for E-33,34

<u>Group</u>	<u>Rat No.</u>	<u>Chemistries (wks)</u>	<u>Hematology (wks)</u>
1M	83616	13 & 26	52
	83613	13 & 26	52 & 104
	83612	13 & 26	52 & 104
	83618	52	104
	83614	13 & 26	52
2M	83731	13 & 26	52 & 104
	83732	13 & 26 (52) ¹	104
	83737	52	104
3M	83811	13 & 26	52
	83813	13, 26, & 52	104
	83814	13, 26, & 52	104
4M	83891	13 & 26	52 & 104
	83893	13, 26, & 52	104
5M	83972	13, 26, & 52	104
1F	83672	13, 26, & 52	104
	83674	13, 26, & 52	104
2F	83771	13, 26, & 52	104
	83773	13, 26, & 52	104
3F	83854	13 & 26 (52) ¹	104
	83855	13, 26 & 52	104
4F	83931	13 & 26	52 & 104
	83932	13 & 26 (52) ¹	104
	83933	13 & 26 (52) ¹	104
	83938	52	104
5F	84011	13	13, 26, & 52
	84016	26 & 52	104
	84017	52	104

¹ At 52 weeks this rat was not sampled for either hematology or clinical chemistries
Rat 84011 was sampled for both hematology and clinical chemistry determinations at week 13

In the process of validating Appendix Table No. 3, E-33 (pp 32-47), UAREP detected a few unimportant discrepancies which are summarized in Appendix IV-10. There were no transcriptional errors noted between the earliest data source and the Entry Book data. Of the 420 means and standard deviations which are recorded in Appendix Table No. 3 of the Entry Book E-33, pages 32-47, 13 inconsequential rounding discrepancies were noted, all of which were in the standard deviation values. Eleven statistically significant differences were reported in Appendix Table 3 of Entry Book E-33,34, pages 32-47, each of which was confirmed by UAREP t-test. UAREP found seven of the eleven significant differences reported not to be significant when based on results of the Analysis of Variance at the 5% level of probability (Appendix IV-11). Thus, the computations which Hazleton performed on these data were carried out with a high degree of accuracy.

Appendix IV-12 shows the confidence intervals for clinical chemistry data indicating the range outside of which values would have to fall to be statistically significantly different from the controls for each interval evaluated. This is based on the variance found within the control groups. All ten mean bilirubin values at 52 weeks are less than 20% those at 13 weeks. This suggests a technical problem.

Hazleton's statistical evaluation of data consisted only of evaluation of interactions between treatment groups and the control groups. UAREP analyzed data for all possible interactions between groups and found additional significant statistical interactions at the 5% level of probability, as given in Appendix IV-11 and 14.

UAREP was constrained by the terms of its contract from commenting on the Searle experiment design. Therefore, we did not consider the question of the size of groups one would need to have in order to detect trends or significant differences in clinical chemistry or hematologic parameters which show substantial inherent individual variation.

Electrophoresis: Hazleton Project Sheet No. 1 and 2 dated December 4, 1969, and Searle protocol dated October 6, 1971, both specified that electrophoresis was to be determined at the 13th, 26th, 52nd, and 104th week intervals on five rats from each of the treatment and control groups. The same rats used as the blood source for the clinical chemistries (glucose, BUN, SGOT, SGPT, alkaline phosphatase) were also used for the electrophoresis determinations.

UAREP validation of Entry Book E-33, Appendix Table No. 3 - Electrophoresis (pp 48-63) revealed a number of discrepancies as summarized in pieces of data transcribed from the laboratory work sheets. Two hundred and forty means and standard deviations were computed by Hazleton and reported in Appendix Table 3 of Entry Book E-33, pages 48-63. UAREP found two computational discrepancies caused by an error made in transcribing data and nine inconsequential rounding discrepancies, all of which were involved with standard deviations.

Appendix Table 3-Electrophoresis (E-33, pp 48-63) indicated that there were 15 significant ($P < 0.05$) comparisons out of the 192 comparisons made between treatment groups and controls. Of these 15 significant t-tests reported by HLA, UAREP data confirmed 11 significant t-tests and two other values were close to the 2.306 required, being 2.25 and 2.20 (Appendix IV-14). UAREP's statistical analysis determined that nine of the 15 reported significant comparisons which were based on the t-test were not significant at the ($P < 0.05$) level of probability based on Analysis of Variance and Least Significant Difference. UAREP performed statistical analysis based on Analysis of Variance, Least Significant Difference, and Newman-Keuls at the 5% level of probability for all possible comparisons between treatment groups which were not computed by Hazleton. UAREP's list of significant comparisons is given in Appendix IV-14. The computed confidence intervals ($P < 0.05$) are shown in Appendix IV-15. Based on the normal control values, these show values which fall outside the 95% range.

At the 13th week interval, a number of the samples used for the determination of the total protein were indicated to be either hemolyzed or of insufficient quantity to perform a determination. Seven of the 25 samples collected for the males at the three week interval were indicated to be hemolyzed. Of the 25 samples collected from the various groups for the females at the 13 week interval, three were quantity not sufficient (QNS) and six were hemolyzed.

Serum Sodium, Calcium, Potassium, Chloride, and Carbon Dioxide: Hazleton Project Sheet No. 1 and 2 dated December 4, 1969 and Searle protocol dated October 7, 1971 both specified that serum sodium, calcium, potassium, chlorine, and carbon dioxide were to be determined at the terminal intervals for all groups. Appendix Table 3 (E-33, pp 64-67) summarizes the results as requested in the experimental design.

UAREP noted a number of discrepancies in the data as reported in Appendix Table 3 of Entry Book E-33, for serum sodium, calcium, potassium, chloride, and carbon dioxide, which are summarized in Appendix IV-16. Of the 250 entries made from the earliest data source, no transcribing errors were noted by UAREP. One hundred means and standard deviations were computed by Hazleton and reported in Appendix Table III of Entry Book E-33,34, pages 64-67. UAREP found two computational errors and five inconsequential rounding errors in standard deviations recorded in that part of Appendix Table 3, relating to serum sodium, calcium, potassium, chloride, and carbon dioxide levels. The Entry Book reported four significant ($P < 0.05$) comparisons between the treatment groups and the control groups, all of which were confirmed by the UAREP t-test. The UAREP validation by different methods found three not to be significant at the 5% level of probability. Significant comparisons between all groups as performed by UAREP including nine not reported in Entry Book E-33,34 (Appendix Table No. 3) are given in Appendix IV-11.

The high and low values for the various parameters determined by Hazleton are compared with the mean and range of normal values from a reference source in Table 4-7. Comparing clinical laboratory values for rats maintained under different conditions can be misleading. However,

Table 4-7
Entry Book E-33, Pages 64-67 Values for Na, K, Cl, Ca and CO₂
at 104 Weeks and Normal Values & Ranges

Entry Book			Reference Source ¹		
<u>Sex</u>	<u>Parameter</u>	<u>Range</u>	<u>Mean</u>	<u>Age of Rats</u>	<u>Range</u>
M	Serum Na (meq/l)	144.0-148.8	148.0±0.70	(25mo)	147-149
M	Serum K (meq/l)	3.72-4.28	6.76±0.18	(25mo)	6.5-7.0
M	Serum Cl (meq/l)	106.0-112.0	105.8±4.76	(25mo)	98-110
M	Serum Ca (mg/dl)	9.32-10.06	11.2±0.28	(7mo)	11.0-11.6
F	Serum Na (meq/l)	143.8-144.8	146.2±1.92	(25mo)	144-149
F	Serum K (meq/l)	3.32-3.81	6.48±0.28	(25mo)	6.1-6.9
F	Serum Cl (meq/l)	102.1-105.1	106.0±2.54	(25mo)	103-109
F	Serum Ca (mg/dl)	10.0-10.25	11.0±0.26	(7mo)	10.8-11.4

¹ Vondruska, J. F., Greco, R. A. Certain Hematologic and Blood Chemical Values in Charles River cd Albino Rats. Bull. Amer. Soc. Vet. Clin. Path. 2: 3, 1973

these data suggest the HLA rat values for both males and females are somewhat elevated for serum calcium, lowered for sodium and chloride, and greatly depressed for potassium. Computed confidence materials based on control values are shown in Appendix IV-17. For the four groups of male rats receiving aspartame, the mean values were at or above the confidence interval for serum calcium in four groups, sodium and potassium in three groups and carbon dioxide in two groups.

L-phenylalanine: Initial Hazleton Project Sheet No. 1 and 2 dated December 4, 1969 and Searle protocol dated October 7, 1971 did not request the determination of L-phenylalanine at any interval. Searle protocol amendment dated December 7, 1971, which was approximately one week prior to the termination of the experiment, requested that 1 ml samples of serum be provided Searle Bioanalytical Lab from five males and five females from Groups 1, 4, and 5 at the terminal interval. Hazleton did not amend their project sheet, but indicated in an internal memo dated December 3, 1971, that 1 ml of serum was to be provided to Searle from each of the five males and five females from Groups 1 and from five males only from Group 5.

Entry Book Appendix Table No. 3 (p 68) indicated that L-phenylalanine levels in the serum for Groups 4 and 5 males were found to be significantly ($P < 0.05$) lower as compared to the controls. The Results and Discussion section of Entry Book E-34 makes the following statement concerning the L-phenylalanine determinations: "Serum L-phenylalanine values were significantly low and serum sodium values were significantly high for Group No. 4 and Group No. 5 males at termination." This significant decrease was confirmed by all methods used by UAREP

(Appendix IV-11) including the computed confidence intervals (Appendix IV-18). Since the monitoring of L-phenylalanine in the serum was to indicate the breakdown of aspartame in the digestive tract to L-aspartic acid and L-phenylalanine and its absorption, some might wonder why additional comments were not included indicating reasons or explanation as to why L-phenylalanine was lower in the treatment groups when it would have been expected to have a higher level. Based on the dosage level of aspartame in the diet of the various treatment groups, one might have expected to see a dose response with respect to the L-phenylalanine levels in the serum. If animals are fasted more than a few hours, the serum phenylalanine levels would be expected to fall in all groups. It would have been of interest to compare the terminal values for L-phenylalanine with determinations at earlier intervals.

Urinalysis - The Searle and Hazleton experimental designs agreed with respect to urinalysis intervals and parameters to be measured. UAREP noted no transcriptional errors in E-33 Appendix Table IV (pp 70-76) as compared to Hazleton's laboratory urinalysis worksheets. The nature of the urinalysis data does not lend itself to statistical analysis, because urine was pooled and much of the data is presented in a non-numerical form.

Ophthalmoscopic Examination

Hazleton Project Sheet No. 1 and 2, dated December 4, 1969, and the Searle protocol dated October 6, 1971, both specified that eye examinations were to be performed at the following three intervals: initially,

at one year, and at the termination of the experiment. Ophthalmoscopic examinations were performed at the following four intervals: initially, 42 weeks, 52 weeks, and terminally (104 weeks), with the summary of these examinations and observations given in Appendix Table No. 5 (pp 79-81). Four HLA internal memoranda dated December 12, 1969, October 8, 1970, December 14, 1970, and January 4, 1972, summarized the eye examinations. UAREP noted no discrepancies in Appendix Table No. 5 of Entry Book E-33.

Necropsy; Body and Organ/Body Weight Ratios

The dates on which necropsies were performed on surviving rats are shown in Table 4-8. For reasons which are not explained in the Entry Book, the survivors for Group 5 females were all sacrificed on December 2, 1971. The rats in all of the other groups underwent terminal sacrifice December 14-20. Although there was some variation in the dates of sacrifice in the various groups with more of the animals in Group 1 being sacrificed later than animals in a number of the other groups, this would present little distortion of results. Two of the females in Group 1 died on December 16 and December 20. For purposes of determining survival time, their survival, however, was considered to be the same as all of the other animals except for the Group 5 females.

Body weights and weights on specified organs were determined on all animals sacrificed at the termination of the experiment. A summary of variation in listing of the organs to be weighed as shown in the Hazleton Project Sheet No. 1 and 2 dated December 4, 1969, Project Sheet No. 4 dated April 30, 1970, Searle protocol dated October 6, 1971, individual animal necropsy sheets, and what was actually weighed at the time

Table 4-8

Dates on which Varying Numbers of Surviving Male (M) and
Female (F) Rats Were Sacrificed

<u>Date</u>	<u>Group 1</u>		<u>Group 2</u>		<u>Group 3</u>		<u>Group 4</u>		<u>Group 5</u>	
	M	F	M	F	M	F	M	F	M	F
12/02/71	0	0	0	0	0	0	0	0	0	10
12/14/71	5	5	5	5	5	5	5	0	5	0
12/15/71	5	5	5	5	5	5	5	5	0	0
12/16/71	5	5	5	5	5	0	5	5	5	0
12/17/71	0	0	3	8	6	10	0	4	11	0
12/20/71	8	11	0	0	0	0	8	0	0	0
Total Sacrificed 12/2 - 12/20	23	26	18	23	21	20	23	14	21	10
Death After 12/16 - 12/20	0	2	0	0	0	0	0	0	0	0

of necropsy, is given in Table 4-9. Spleen weights were requested in Hazleton Project Sheet No. 1 and 2 and 4 and are shown on the "single animal autopsy sheet." The Searle protocol dated October 6, 1971, which was duplicated in the Hazleton Project Sheet No. 6 dated November 4, 1971, superseded the instructions contained in the Hazleton Project Sheet No. 1 and 2 and 4, and the spleen was not weighed at the time of necropsy. E-34 indicates that the thyroid and adrenal were weighed after fixation, but this is not shown in the protocols. The Searle protocol appears to have specified that the mammary tissue (R 4 and 5) was to be weighed at the time of necropsy. This was crossed out on HLA Project Sheet No. 6, was not done, and would have been of variable accuracy.

UAREP validation of organ and organ/body weight ratios found discrepancies in Appendix Table No. 1 of the Entry Book E-33, which are summarized in Appendix IV-19. UAREP encountered difficulty in its initial validation of terminal organ and body weights because in a number of groups the number of animals involved did not agree with the "N" value utilized by Hazleton. Although not discussed in E-34, UAREP eventually learned from HLA that animals which had tumor masses greater than 10% of the body weight were deleted from computations of organ to body weight ratios since it was presumed that the mass of their tumor might produce abnormal variation in organ to body weight ratios. Although weights from tumor masses were not available in any of the experiments reviewed by UAREP, a few of the rats in E-70 were also deleted from organ/body weight ratio from computations for the same reasons. No such deletions, however, were made in E-75 or E-76 which used mice. Dr. Reno claimed that none of the mice developed tumors

Table 4-9

Comparison of Background Information and Entry Book Data on

Organs Weighed at Necropsy

	<u>HLA Project Sheets</u> <u>12/4/69</u>	<u>4/30/70</u>	<u>Searle Protocol</u> <u>10/6/71</u>	<u>Individual Necropsy Sheets</u>	<u>Data in Entry Book</u>
Heart	X	X	X	X	X
Kidney	X	X	X	X	X
Liver	X	X	X	X	X
Thyroid	X	X	X	X	X
Adrenal	X (added)	?	X (added)	X	X
Gonad (ovary; testis)	X	X	X	X	X
Uterus	X (added)	?	X	X (write in)	X
Prostate (M)	X (added)	?	X	X (write in)	X
Seminal Vesicles	X (added)	?		X (write in)	X
Mammary (F)			X		
Spleen	X	X	(blot out?)	X (crossed out)	
Urinary Bladder	X	X?		X (crossed out)	

Some variation is noted in information sources specifying (X) organs to be weighed

equalling 10% of their body weight. UAREP had no HLA data to check on this although it is recognized that mouse tumors occasionally grow to more than 10% of the body mass.

UAREP was unable to determine from the protocols or writeup in E-34 precisely how HLA derived their significance values in E-34, Figure No. 4, summary of Table No. 7. The comment on page 14 of E-34, says that Analysis of Variance was to be used. They reported 13 significant differences between the means. UAREP agreed on all 13 with their t-test (Appendix IV-19A). On three of the 13, our ANOVA values were .17, .23, and .44 which were not close to $P < 0.05$. In addition to the 13 concurrences, UAREP had ten additional significant t-tests for which HLA analyses were not significant. ANOVA and t-tests are not testing for significance in the same manner and could be expected to get different results at times. Such differences are illustrated throughout this UAREP review. Although the numbers used for weights for HLA and UAREP were in close agreement, the HLA data on significance disagreed at times with both UAREP Analysis of Variance and t-tests. However, neither HLA or UAREP proposed any biologic significance of these statistically significant differences in means.

Histopathologic Findings

UAREP's primary objective was to assess the accuracy of the E-33,34 report relative to the recognition, interpretation (diagnosis) and grading of severity of significant pathologic processes on a scale of 1 to 5. As appropriate, we would make an independent assessment of whether significant disease processes were related to treatment.

Secondary objectives included: (1) determining the precision with which protocol specifications had been followed with respect to fixation and sectioning of tissues for examination, and (2) correlating clinical observations, gross necropsy findings, and histopathologic diagnoses to assess the possibility of failure to examine microscopically important tissue lesions.

Those responsible for preparation of the UAREP report encountered certain problems in their attempts to ascertain whether the EPL and UAREP pathologists were looking at the same lesion, and more significantly interpreting it in terms with equivalent diagnostic meanings. One such problem in diagnostic terminology relates to proliferative lesions of the liver in rats. As mentioned in Chapter II, there has been recent progress in improving and standardizing the terminology of such liver lesions. The criteria employed 10 or 20 years ago for diagnosing hepatomas and hyperplastic or regenerating nodules, were certainly not universally accepted then. In general, many pathologists used the terms "hyperplastic nodule or regenerating nodule" to describe a proliferating lesion which they did not think had yet attained the fully established status of a neoplasm. Some felt that a certain percentage of such nodules might eventually become tumors. The term, hepatoma, on the other hand, was formerly used by many rodent oncologists to cover both benign and malignant neoplasms of hepatic parenchymal cells.

In this UAREP study, the following more current terminology (Squire, R. A. and Levitt, M. H., reference 10 in Chapter II) was utilized because it gives a more precise definition of lesions:

- (1) Focus or area of change - a non-neoplastic or pre-neoplastic change in parenchymal cells less or more than one lobule in size, respectively, which may have eosinophilic, basophilic, or clear cytoplasm; and does not compress the surrounding parenchyma.
- (2) Same as (1) but is accompanied by compression of surrounding parenchyma.
- (3) Hepatocellular carcinoma - malignant neoplasm arising in hepatic parenchymal cells.

In the previous literature some foci or areas of change, and neoplastic nodules would often be termed regenerative or hyperplastic nodules, while other neoplastic nodules and hepatocellular carcinomas might be termed hepatomas. In the table of discrepancies (Appendix IV-26) these differences in terminology are noted with an asterisk.

In the original Searle report several renal lesions were noted to have a higher incidence in the very high level males. These included focal hyperplasia of renal pelvic epithelium and tubular degeneration. In the UAREP review, these differences were confirmed. A complex of renal lesions including interstitial inflammation, fibrosis, tubular degeneration and regeneration and glomerulosclerosis are often observed in older rats (sometimes called "old rat nephropathy"). Such changes were indeed present in all rat groups of the present study. The very high dose males, however, also had an increased amount of tubular basophilia, small areas of tubular hyperplasia, and areas of

pelvic epithelial hyperplasia with atypia. Whether or not such changes might be premalignant cannot be answered from the available data.

Comparison of EPL and UAREP Histopathologic Diagnoses

The conditions under which the comparison of histopathologic diagnoses were made are described in some detail in Chapter II in the section entitled, "Review of Histopathology Diagnoses." For comparative purposes, the diagnoses of neoplastic and non-neoplastic conditions in these experiments will initially be considered separately as was done by EPL.

Tumors - UAREP pathologists devoted special attention to neoplasms. All tumors were evaluated with respect to type, malignancy, and extent. Whenever a discrepancy in a tumor diagnosis occurred between UAREP and EPL, the tissue section was re-evaluated according to the procedure discussed in Chapter II.

A tabulation of tumor frequencies is recorded in Appendix IV-20. The table gives the total numbers of tumors according to histologic types and site of occurrence, and shows a comparison between UAREP and EPL results by groups. The UAREP method of tabulation in Appendix IV-20 differs somewhat from that used in the original EPL report in E-34, Figure No. 5, pages 45-50, "Frequencies of types of histologically proven tumors in male and female albino rats receiving SC-18862 or serving as controls." This results in different total numbers for some tumors. UAREP felt that each tumor should be counted only once, and therefore did not count metastatic lesions or each organ site involved

by generalized tumors such as the lymphomas as more than one tumor. To avoid confusion and guesswork as to the primary site of the lymphoreticular tumors, the total number of animals showing such tumors are listed under the heading "All Organs" rather than the individual organs involved. All lymphoreticular and hematopoietic tumors, including lymphomas, lymphosarcomas, reticulum cell sarcomas, leukemias, and myelomas are grouped together under the term "lymphoma" to facilitate ease of counting and classification.

When multiple tumors of the same type occurred in the same animal, such as several fibroadenomas or adenocarcinomas of the mammary glands, each tumor diagnosed in the EPL diagnoses sheets was counted individually, whereas the UAREP pathologists diagnosed only one tumor of any particular type in any specified organ. When UAREP pathologists reviewed slides and were not conversant with the details of procedures followed by EPL and HLA, they could not always be certain that they were not looking at the same tumor in different sections of a single tissue mass. Although UAREP pathologists making diagnoses did have access to the necropsy reports, correlation between gross findings and multiple tumors on the histopathology slides was often not attempted. This also contributes to discrepancies between EPL and UAREP tumor totals. Taking these factors into account, there appears to be good correlation between the total numbers of tumors reported by the EPL and UAREP pathologists. However, the totals do not show individual discrepancies in tumor diagnoses; these are listed with all the histopathology discrepancies in Appendix IV-26.

There is good agreement between EPL and UAREP as to the number of sections of each organ examined (Appendix IV-20). The differences in numbers of brain sections in Groups 2, 3 and 4 examined by EPL and UAREP relates to the fact that after EPL concluded their review for E-33,34, Searle requested that brains of all rats in all groups be sectioned to search for brain tumors. After Dr. Innes had reviewed these sections and completed his report (see Chapter IX), all the brain sections were combined with other sections of each rat.

Appendix IV-20 shows figures for average number of any type of tumors per animal. These figures were obtained by dividing the total number of rats in a group by the total number of tumors as counted by the UAREP method (explained in footnote to Appendix IV-20). This would be one broad measure of carcinogenic action. There is general agreement between UAREP and EPL figures and there is no evidence of a dose related difference between controls and aspartame fed rats.

A complete listing of tumors diagnosed by UAREP is given in Appendix IV-21. For the reasons already stated, the lymphoreticular and hematopoietic tumors are not listed under individual organ sites by UAREP in Appendix IV-21 and are grouped under the one heading "lymphoma."

In all experiments of E-33,34 and E-70, a few sections of organs were lost and not available to UAREP to examine, but few of these had significant diagnoses and they were not selectively from controls or experimental groups. HLA did succeed in finding for UAREP a number of slides of livers and brains which had tumor diagnoses. Seven slides which had a tumor diagnosis of EPL were missing and not available for diagnosis by UAREP. The distribution of these few slides in terms of

organ diagnosis and experimental group would not have significantly altered any UAREP conclusions had they been present (Appendix IV-22). Instances in which UAREP or EPL indicated a slide as missing and the other made a diagnosis, were not considered discrepancies in Appendix IV-26.

Statistical Analysis of Tumor Incidence: As stated in Chapter II, UAREP applied a modified life table analysis technique to the tumor incidence based on UAREP diagnoses as shown in Appendix IV-21. Analysis was made comparing each of the five groups with the other four groups individually, as well as with Groups 2 and 3 combined and in Groups 4 and 5 combined. The tumors were analyzed by UAREP according to the categories of: any tumor, benign tumors, malignant tumors, adrenal cortical tumors, adrenal medullary tumors, pituitary tumors, all mammary tumors and malignant mammary tumors. This means that the eight different categories of tumors were each subjected to 12 comparisons for males and for females at $P < 0.05$. Since some groups were without any tumors of a specific type there were 170 group comparisons (instead of 192) of which two were significant.

The male Group 1 vs Group 2 rats had a $P < 0.05$ for pituitary tumors. It is not surprising that the difference in the incidence of pituitary tumors in Group 1 males vs Group 2 males should be statistically significant since 13 of the 60 males in Group 1 developed pituitary tumors and only two in 40 males in Group 2 developed pituitary tumors. The male Group 2 with eight tumors vs Group 3 rats with one

tumor had a $P=.03$ for adrenal medullary tumors. There were no significant differences in the cumulative incidence rates for tumors in female groups.

The remaining 168 of the 170 comparisons had probability values greater than 0.05. In making 170 comparisons, one might expect to get eight values at the 5% level by chance alone. The two significant values are less than one would expect. The life table method of analysis takes into account the time of onset of each tumor. Time of onset of course, is a misnomer since we are only able to ascertain the time at which the tumor (or some other process) produced death of the animal or in the case of superficial tumors, the time it could first be palpated. This assumes, incorrectly, that those animals sacrificed terminally had pituitary tumors which had been growing for an equivalent length of time to that in animals which died with pituitary tumors prior to the termination of the experiment. The life table method takes into account the onset of each tumor, and the number of animals at risk for various intervals. This method is better than using a single criterion such as the percentage of animals developing tumors or the mean number of tumors per animal.

UAREP does not feel that the evidence is sufficient to warrant a conclusion that the feeding of aspartame had any direct relationship to the production of pituitary tumors. In the male rats, the controls had a significantly higher incidence than the Group 2 low dose animals. There was no pattern of consistent compound or dosage related incidence of pituitary or adrenal medullary tumors in E-33,34 and likewise, there

was no evidence of such a relationship in the other rat experiment reviewed by UAREP, E-70. UAREP made no attempt to differentiate between the relative incidence of benign and malignant pituitary tumors, as this is difficult and as mentioned in Chapter II, the evidence for malignancy of pituitary tumors is controversial.

In Figure No. 7, page 67, of E-34, Hazleton reports the number of proven histologic tumors in male and female albino rats receiving aspartame or serving as controls. Generally, there is reasonably close agreement between UAREP and Hazleton in the total number of tumors diagnosed, as shown in Appendix IV-23. Likewise, there is generally close agreement between the total number of tumors in the categories for which Hazleton and UAREP both analyzed probabilities of tumor incidence. Hazleton did not analyze for adrenal cortical tumors, adrenal medullary tumors, or pituitary tumors.

In Figure No. 8 (E-34, p 68), Hazleton presents their data for probability of tumor incidence. These figures are compared with UAREP figures in Appendix IV-24. The values for the probability of tumor incidence are based on the life table analysis method. Both UAREP and HLA used methods for life table analysis which were presumed to be essentially identical. The final cumulative incidence figures at 104 weeks are those presented in Appendix IV-24. When we compared the results at intervals before week 100, we generally found very close agreement between the results of HLA and UAREP. As far as we could ascertain, our input data for analysis was very similar to that used by HLA. Because of the consistent difference in terminal values, UAREP decided it was not worth further time to attempt to determine precisely what HLA had done to achieve different final results.

Of more importance than the figures on probability of tumor incidence is the analysis of the statistical significance of these figures. Unfortunately, UAREP is not clear as to the methods of analysis of the HLA tumor data. The Entry Book states that they applied their life table analysis which was followed by a t-test. Details as to the application of this t-test are not stated. In their analysis of significance of the tumors in males, Hazleton reported $P < 0.05$ for any tumor for Group 1 vs 3; for benign tumors for Group 1 vs 3; and for malignant tumors for Group 1 vs 5. None of these comparisons were significant by UAREP analysis, although as stated earlier, pituitary tumors were decreased in Group 2 as compared with control males and adrenal medullary tumors in Group 2 as compared with Group 3 males. Neither Hazleton nor UAREP found any significant differences in tumors in female groups. Despite the differences noted, both the E-34 report and UAREP agree that there is no evidence of significant biologic differences in tumors in these experiments.

Non-neoplastic Diagnoses - The significant diagnoses of non-neoplastic lesions made by EPL and UAREP are tabulated and compared in Appendix IV-25 A, B, C, and D. The EPL data is taken from E-34, Figure No. 9 and 9A, pages 70-97. It should be noted that some of the diagnoses listed by EPL were eliminated by UAREP and that others were combined or reworded. To facilitate comparing data the EPL diagnoses were reclassified into UAREP's terms. It should be stressed that only unimportant diagnostic categories were eliminated and that no potentially significant diagnoses were changed. It should also be noted that UAREP

included endometrial polyp as a diagnosis under "uterus" in the table listing the non-neoplastic lesions, as did EPL (Appendix IV-25). For completeness, UAREP also included the endometrial polyps in the listing of tumors; however, EPL did not.

The diagnoses as given indicate neither whether the diagnosis was made on the same or on different animals by UAREP and EPL nor the grading of the severity of lesions on a scale of 1 to 5. The number opposite the organ indicates the number of sections examined by UAREP and EPL. Although the agreement in diagnoses by EPL and UAREP is remarkably good, there are discrepancies in total numbers of specific diagnoses which indicate the differing inclinations of pathologists to make these diagnoses. For example, in the spleen EPL diagnosed extramedullary hematopoiesis in 66% of all rats, 9 times as frequently as UAREP, but seldom diagnosed reticuloendothelial cell hyperplasia which was reported 25X as frequently by UAREP. Rat spleens are very complex mixtures of cells, and elements of the two diagnoses could be confused. EPL diagnosed focal fibrosis in 78% of the hearts examined, which was 3X as often as UAREP. The fact that UAREP's lung diagnoses were more frequently tabulated under acute inflammation and EPL's under abscess of the lung, is an example of differences in terminology used by pathologists in looking at the same lesions.

Discrepancies in Histopathologic Diagnoses - Those discrepancies in individual histopathologic diagnoses considered potentially significant are shown in Appendix IV-26. Most unimportant diagnoses have been

deleted from this list as well as those for which one might expect a considerable difference in the number of pathologists choosing to record a diagnosis even though its presence was recognized. Minor differences in grading the severity of lesions likewise were not included in this discrepancy list. According to the definitions in Chapter II, about half of these discrepancies could be classified as major of which half involve the distinction between a neoplasm and a non-neoplastic proliferative hyperplasia. This is an important distinction, but one that occasionally requires the pathologist to draw a fine line of demarcation. They do not all agree on such diagnoses all of the time.

In connection with discrepancies listed in Appendix IV-26, UAREP feels that there is generally good agreement between the diagnoses arrived at by EPL and by UAREP's Maryland pathology reviewers. There were, after all, nearly 14,000 tissue sections reviewed in this E-33,34 experiment and many of the sections were subject to multiple diagnoses. UAREP and EPL agreed on all major diagnoses not listed as discrepancies in Appendix IV-26. All pathologists occasionally fail to observe a lesion or interpret one somewhat differently from their colleagues.

From their years of experience, EPL has developed a good system for verifying their histopathologic diagnosis tables, thereby reducing transcriptional errors. In the material under review by UAREP, which was among the first major projects undertaken by EPL, it is evident that transcriptional errors did occur, that is, a diagnosis was entered in the vertical column (for an animal) or on a horizontal line (for a diagnosis) adjacent to that which was probably intended. UAREP constructed a transparent grid to be superimposed upon the EPL diagnoses

tables to facilitate lining up the vertical and horizontal axes to the point to which EPL diagnosis was entered in Table 8.

UAREP pathologists had a number of advantages over the original EPL histopathologists. As the UAREP diagnoses were eventually matched against comparable EPL diagnoses, occasional transcriptional errors made by UAREP became evident and were then corrected. All possible discrepancies in diagnosis were reviewed by a panel of three or more pathologists who were unaware of the EPL diagnosis. When a final consensus diagnosis was reached, it was compared with the EPL diagnosis. If at that time the difference was only one of nomenclature, the term used was changed to that used by EPL except in the case of the liver for reasons given above.

Disease Related to Treatment - UAREP confirmed some non-neoplastic lesions in the kidneys of Group 5 males as described earlier under Histopathologic Findings. This agrees with the E-33,34 report. UAREP found no other conditions which appeared to be treatment or dose related.

Discrepancies in Following Protocol Design and Correlation of Clinical vs Gross Necropsy vs Microscopic Observations

Findings of all animals were reviewed from the INTEC printouts of clinical observations, with emphasis being placed on palpable nodules and masses that were present at the last clinical examination prior to death or sacrifice of the animal. In those instances in which lesions were noted clinically, the necropsy reports were carefully checked for

recording and fixation of comparable lesions by the prosector. From the histopathology incidence tabulations (a listing of tissues cut and slides prepared for each animal), we verified that these lesions were sectioned and microslides prepared. The existence of a subsequent appropriate diagnosis was sought in the tables of detailed histopathologic microscopic findings prepared by EPL (E-33, Table No. 8, pp 100-342).

It was not expected that all the lesions observed clinically would prove to be tumors nor was it expected that all the nodular lesions described would be represented by a diagnostic listing under the heading "tissue mass." Diagnoses listed under appropriate organs were assumed to be evidence that the clinically described lesions had been examined microscopically. Concurrently, the necropsy sheets (see Appendix IV-30) were evaluated for completeness. The listing of tissues preserved and the tables showing sections cut were checked against the protocol to verify adherence to the guidelines set down in experiment design. Any gross lesion in any internal organ described at necropsy was followed through the sequence described to ascertain that that lesion had been examined microscopically.

In general, UAREP noted excellent correlation between the clinical, gross, and microscopic findings recorded in the report and adherence to the protocol guidelines, insofar as the histopathology is concerned. Considering the number of animals used in the experiment, the volume of data involved, and the enormous number of slides prepared, some discrepancies are to be expected. None of these, however, affected the

findings of the experiment or detracted from the significance of the experiment results. The types of discrepancies noted are:

- a) A gross lesion was described clinically or at necropsy, without a comparable microscopic diagnosis recorded.
- b) A microscopic section of a tissue was apparently prepared, according to HIT (a tabulation of sections prepared) but no diagnosis was listed for that organ. It is probable that the tissue proved to be unremarkable upon microscopic examination, but the "X" (unremarkable) designation was omitted from E-33, Table No. 8. If no lesion was noted grossly in that particular tissue, UAREP considered this type of discrepancy to be of minor importance, unless UAREP pathologists diagnosed a significant lesion upon their review of that particular slide.
- c) Conversely, some instances were noted where EPL listed "N" (no section) in the diagnoses Table No. 8, but showed that a slide had been prepared in the HIT. This was also considered to be of minor importance unless the UAREP reviewers diagnosed a significant lesion in that slide.
- d) In a few instances, the total number of slides prepared from a particular animal was incorrect, according to the listings of the individual sections cut. This kind of discrepancy has no significance to the study, but does show some degree of inaccuracy in some aspects of the research work.

A listing of these discrepancies for Group 1 of the experiment is shown in Appendices IV-27, IV-28, and IV-29. Considering the amount of work involved, and the minor importance of the discrepancies discovered, UAREP deemed it unnecessary to create similar tables for the remainder of the groups.

CONCLUSIONS

This is a relatively large 104 week experiment, utilizing 440 rats, with four different levels of aspartame in the diet. Five hematologic, 15 clinical chemical and seven urinalyses parameters were measured, many at four or five intervals throughout the experiment. Many of the clinical laboratory determinations were made on groups of five male and five female rats. For most rats, this was a lifetime study, which began shortly after weaning. Because of the fact that many rats died and because rats initially used for clinical chemistry blood samples were subsequently used for hematology sampling, it was not possible to sample the same animals throughout the experiment. In fact, to obtain the ten samples for clinical chemistries at the various intervals, it was necessary to use fifty different animals over the course of the experiment.

The format of the Searle protocol for this experiment was substantially neater in appearance than that used for E-28 in Chapter III. The design of the experiment underwent numerous changes as it progressed, presumably partially due to the experience gained in the earlier phases of the experiment. It was not feasible for UAREP to reconstruct the evolution of the experiment by finding matching written documentation of changes by Searle and Hazleton Laboratories. The written documentation provided to UAREP suggests that the experiment had been underway for many months before the Hazleton Project Sheets correctly indicated the

number of groups of animals, the number of animals in the group, and the procedures to be carried out when animals died. In fact, the two year experiment had been underway for 23 months before the Hazleton Project Sheets reflected the procedures to be carried out following necropsy, as they were indicated in the Searle protocol earlier.

Searle protocols and Hazleton Project Sheets were vague as to the statistical procedures to be employed in analyzing data. The Entry Book provided some additional information but specific details were lacking as to the precise procedures undertaken for all of the various types of statistical analyses. Since UAREP could not always be sure of the methods or conditions under which the statistical analysis had been carried out, we applied not only a t-test and life table analysis, but also Analysis of Variance, Least Significant Difference (which had been used by Searle in E-28), and the Newman-Keuls test as a more critical test of significance. UAREP also chose to compare all treatment groups with each other, whereas HLA compared each treatment group only with the controls. The overall result of UAREP's statistical analysis was to agree with HLA on many of their t-tests, and to show an overall lower incidence of the statistically significant comparisons. The comparison of multiple treatment groups at different dosages also confirmed the absence of dose-related effects of the aspartame.

UAREP carried out a somewhat more detailed analysis of body weight changes than HLA. UAREP found that female groups 1, 2, and 3 weighed significantly more than the very high treatment group for weeks 12-84 and that similarly, Group 4 weighed more than the very high treatment

group for weeks 26-84. The very high treatment group of males weighed less than the other four treatment groups for weeks 26-104.

UAREP not only confirmed Hazleton's finding of significantly decreased food intake in those rats eating the most aspartame, as compared with the controls during the first year, but also demonstrated that the rats consuming the highest levels of aspartame tended to eat less than those eating lesser amounts of aspartame, and that these changes generally carried on through the second year.

There are technical problems in being certain that rats are fed precisely the amount of aspartame desired on a body weight basis per day. Nevertheless, the results presented lead one to believe that the fluctuations in compound consumption average out reasonably well over a long period of time so that one has an adequate opportunity to study differences in dose response. The fact that Hazleton ran out of aspartame on two occasions for a few days would not significantly affect the course of long-term experiments.

For unexplained reasons, the female rats receiving the highest dosage of aspartame were sacrificed at 102 weeks instead of at 104 weeks as all of the other rats. Hazleton erred in computing the mean survival time for these animals as 423 days instead of 602 days, because the ten rats which survived 102 weeks were omitted from their computations. There was complete agreement between Hazleton and UAREP on the percentage survival rate data which is derived from the life table analysis. UAREP's life table analysis indicated that Group 4 males had a significantly higher rate than Group 1 and that Group 2 and Group 1 females had significantly higher survival rates than Group 5. Hazleton noted

the statistically significantly decreased survival of the Group 5 females and did not test for the comparison of Group 2 females with Group 5. UAREP noted that there was considerable variability in the hematologic and clinical chemistry results. Many who have worked with such parameters in rats have also noted variability. It is not possible for UAREP to assess how much of this variability related to inherent biologic factors in the rats, variations in methods of collecting, transporting, storing, and analyzing the specimens, or to sample size and experiment design.

UAREP checked literally tens of thousands of bits of data and found that transcriptional errors were rare. Although a number of rounding discrepancies were noted, there are a number of acceptable methods of rounding numbers and it seems probable that Hazleton used a different method than UAREP. All of these inconsequential rounding discrepancies were without any effect on interpretation of results. Computational discrepancies were noted rarely. When UAREP could be sure of using the same methods of statistical analysis as Hazleton, we confirmed most of their findings of statistically significant differences. Our comparisons of all treatment groups with each other and the methods of analysis we used which were not employed by Hazleton, convinced UAREP even more than the original Hazleton findings, that the tests of significance tended to be spurious and did not indicate compound and dose relationships.

UAREP was interested in the determinations of serum L-phenylalanine and confirmed Hazleton's finding of its statistically significant decrease at termination of the experiment. Since aspartame is broken down in the digestive tract to phenylalanine and aspartic acid, we would have

expected the serum level of phenylalanine to be increased, especially in the high dose animals. The determinations were available only for the terminal interval. Noteworthy discrepancies in urinalysis or ophthalmoscopic examination were not observed by UAREP.

UAREP had difficulty understanding the precise statistical analysis methods used by Hazleton for analysis of body weights, organ weights, and organ to body weight ratios. Although there were areas of agreement and disagreement in significance, we concurred that the changes were without biologic significance.

The review of the histopathologic diagnoses was a major undertaking for UAREP. Discrepancies in diagnoses were noted, but because of the large number of specimens involved, it was felt that there was generally good agreement between the EPL and UAREP histopathologic diagnoses. There were no significant increases in neoplastic or non-neoplastic lesions in any of the experimental groups except the renal lesions previously described in Group 5 males. Although UAREP was unable to duplicate satisfactorily all aspects of Hazleton's application of life table analysis to tumor incidence, we both agreed that there was no significant increase of tumors relating to the feeding of aspartame. UAREP analyzed more types of tumors than Hazleton and showed that there was a significant increase in pituitary tumors in male rats as compared with the low dose animals. This was the only statistically significant difference noted by UAREP. UAREP ascertained that Hazleton and EPL followed the specifications of the protocol very closely in carrying

out the weighing of organs, fixing of tissues, sectioning of tissues, and histopathologic review. In UAREP's opinion, the correlation between the clinical observations, gross necropsy, and microscopic observation was quite satisfactory.

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APPENDIX IV-1

SEARLE PROTOCOL, HAZLETON PROJECT SHEETS AND AMENDMENTS

Project Sheets (PS) Supplied by Hazleton

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Items A' to E' are essentially duplicates of corresponding Hazleton items.

Item A

THIS PROJECT SHEET REPLACES ALL PREVIOUS SHEETS. PLEASE DESTROY PREVIOUS PROJECT SHEETS NO. 1 AND NO. 2.

HAZLETON LABORATORIES PROJECT SHEET

850273

PROJECT SHEET NO. <u>1</u>		PROJECT NO. <u>700-233</u>																											
PROJECT COORDINATOR Jessup/Reno		DATE December 4, 1969																											
COMPOUND(S) SC-18862	LOT NO(S). A-3427	RECEIPT DATE 10-29-69	LH-NUMBER(S) 12,237B																										
DIVISIONS PARTICIPATING Toxicology	DISTRIBUTION: CENTRAL FILE (2) EACH DIV. PARTICIPATING EACH DIV. DIRECTOR PROJ. COORD. DATA PROCESSING																												
PHYSICAL AND CHEMICAL PROPERTIES		RECEIVED DEC 8 1969																											
SPECIAL PRECAUTIONS (INDICATE PERSONNEL HAZARDS, PROTECTIVE INSTRUCTIONS)		CHRONIC TOXICOLOGY SECTION																											
REFERENCE INFORMATION																													
PROGRESS REPORTS DUE	FINAL REPT DUE on completion	INITIALS DCJ:sfh	SIGNATURE (PROJ. COORDINATOR) 																										
EXPERIMENTAL WORK to be performed in Small Animal Toxicology Section																													
<p><u>Two-Year Toxicity Study - Rats</u></p> <p><u>Animal Groups</u> - Four hundred twenty weanling albino rats, 210 males and 210 females, will be selected at random and placed into the following groups:</p> <table border="1"> <thead> <tr> <th rowspan="2">Group No.</th> <th colspan="2">No. of Animals</th> <th rowspan="2">Dietary Levels g/kg</th> </tr> <tr> <th>male</th> <th>female</th> </tr> </thead> <tbody> <tr> <td>1 (Control)</td> <td>60</td> <td>60</td> <td>0</td> </tr> <tr> <td>2</td> <td>40</td> <td>40</td> <td>1</td> </tr> <tr> <td>3</td> <td>40</td> <td>40</td> <td>2</td> </tr> <tr> <td>4</td> <td>40</td> <td>40</td> <td>4</td> </tr> <tr> <td>5</td> <td>40</td> <td>40</td> <td>6</td> </tr> </tbody> </table> <p>Group No. 1 will serve as a control group and will be treated in the same manner as the other groups except that no test material will be administered. Group No. 5 will receive 6 g/kg during the first two months. The dose will be increased probably after two months, and the changed dose level will be indicated by the Project Manager at that time.</p> <p>The rats in each group will be individually housed.</p>				Group No.	No. of Animals		Dietary Levels g/kg	male	female	1 (Control)	60	60	0	2	40	40	1	3	40	40	2	4	40	40	4	5	40	40	6
Group No.	No. of Animals		Dietary Levels g/kg																										
	male	female																											
1 (Control)	60	60	0																										
2	40	40	1																										
3	40	40	2																										
4	40	40	4																										
5	40	40	6																										

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Project Sheet No. 1
Project No. 700-233 (850273)

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December 4, 1969

Water and the appropriate diets will be freely available during the course of the study.

Diet and Compound Administration - In order to ensure proper dosing on a g/kg body weight basis during the weeks of rapid growth, the following regimen will be followed:

- Weeks 0 through 4 - Calculate individual food consumption three times per week, and adjust dose according to body weight change.
- Weeks 5 through 13 - Calculate individual food consumption two times per week, and adjust dose according to body weight change.
- Weeks 14 through 26 - Calculate individual food consumption every week, and adjust dose according to body weight change.
- Weeks 27 through 52 - Calculate individual food consumption bi-weekly, and adjust dose according to body weight change.
- Weeks 53 to termination - Calculate food consumption monthly, and adjust dose according to body weight change.

NOTE: In order to conserve test material during the stage of rapid growth do not discard remaining feed every two days or three days, but adjust concentration by addition of diet or test material where appropriate.

Observations - Individual body weights and food consumption will be recorded as indicated above.

Observations of gross signs of toxicity; pharmacological effects; and the incidence, size, and location of tumors will be recorded at the same intervals.

The rats will be observed daily for mortality.

Necropsies will be performed on all rats which die during the course of the study, and tissues will be taken.

Ophthalmoscopic Examination - Will be performed on all animals initially, at one year, and at termination.

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Project Sheet No. 1
Project No. 700-233 (850273)

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December 4, 1969

Clinical Studies - The following clinical studies will be performed on five male and five female animals from the control and each test group:

Hematology - At 13, 26, 52, and 104 weeks:

hematocrit	erythrocyte count	differential leukocyte
hemoglobin	total leukocyte count	count

Clinical Biochemistry - At 13, 26, and 52 weeks:

fasting blood sugar	total serum bilirubin	serum alkaline phos-
blood urea nitrogen	serum glutamic-pyruvic	phatase
total serum protein	transaminase	serum electrophoresis

- At 104 weeks:

fasting blood sugar	serum potassium	serum alkaline phos-
blood urea nitrogen	serum chloride	phatase
total serum protein	carbon dioxide	serum glutamic-oxaloacetic
total serum bilirubin	serum calcium	transaminase
serum albumin	serum glutamic-pyruvic	serum electrophoresis
serum sodium	transaminase	

Urine Analysis - At 13, 26, 52, and 104 weeks (pooled samples):

pH	ketones	microscopic examination
specific gravity	total protein	of sediment
glucose	bilirubin	

*fast feed
wine subs*

- At monthly intervals throughout the study:

phenylketonuria (dipstick method)

pool 5+5

Terminal Necropsy - At 104 weeks the study will be terminated, and the following procedures will be followed:

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850273

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December 4, 1969

POSTMORTEM PROCEDURES

Organ Weights - Indicated in Column I from each animal at terminal sacrifice.

Preservation of Tissues - Indicated in Column II, from each animal, in 10% neutral buffered formalin unless indicated otherwise.

Histopathological Evaluation - From 30 males and 30 females in Groups 1 and 4.
- From 15 males and 15 females in Groups 2 and 3.
- From males and females in Groups .

Tissues	I Wgt.	II Fixed	Histopathology, Group No.						Special Observations or Procedures
			1	2	3	4	5	6	
Brain	✓	X							
Pituitary	✓	X	X			X			
Spinal Cord	✓	X							
Eye	✓	X							
Salivary Gland	✓	X							
Thyroid	✓ X	X	X			X			
Parathyroid									
Thymus									
Trachea									
Esophagus									
Lung	✓	X							
Heart	✓ X	X	X			X			
Liver	✓ X	X	X	X	X	X			
GASTROINTESTINAL									
Spleen	✓ X	X	X			X			
Kidneys	✓ X	X	X	X	X	X			
Adrenals	✓ X	X	X			X			
Stomach		X	X	X	X	X			
Pancreas	✓	X	X			X			

B = both sexes; M = male only; F = female only

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December 4, 1969

Histopathology.

B = both sexes; M = male only; F = female only

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850273

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December 4, 1969

Analysis and Report - At termination of the study, the results will be reported in full giving:

experimental design
general physical appearance
behavior
effects on body weight, food
consumption, and survival

gross signs of toxic or
pharmacological effects
clinical findings
individual gross and microscopic
necropsy findings

- Statistical evaluation:

body weights
food consumption
survival

organ weights
organ/body weight ratios

- Tables will be furnished showing:

mean weekly body weights
weight ranges
food consumption
survival data
individual hematological values
individual biochemical values

results of urine analysis
mean terminal body weights,
organ weights, and organ/body
weight ratios
tissue mass incidence

Mean weekly body weights and food and compound consumption will be presented graphically.

00005

Item B

HAZLETON LABORATORIES PROJECT SHEET

850273

PROJECT SHEET NO. <u>3</u>		PROJECT NO. <u>700-233</u>	
		PROJECT COORDINATOR <u>Jessup/Reno</u>	DATE <u>April 6, 1970</u>
COMPOUND(S) <u>SC-18862</u>	LOT NO(S). <u>A-3427</u>	RECEIPT DATE <u>10-29-69</u>	LH-NUMBER(S) <u>12,2378</u>
DIVISIONS PARTICIPATING <u>Toxicology</u>	DISTRIBUTION: CENTRAL FILE (X) EACH DIV. PARTICIPATING EACH DIV. DIRECTOR		Sponsor PROJ. COORD. DATA PROCESSING
PHYSICAL AND CHEMICAL PROPERTIES			
SPECIAL PRECAUTIONS (INDICATE PERSONNEL HAZARDS, PROTECTIVE INSTRUCTIONS)			
REFERENCE INFORMATION			
PROGRESS REPORTS DUE	FINAL REPT DUE <u>on completion</u>	INITIALS <u>FER:mtg</u>	SIGNATURE (PROJ. COORDINATOR) <u>[Signature]</u>
EXPERIMENTAL WORK to be performed in Small Animal Toxicology			
<p><u>Two-Year Toxicity Study - Rats</u></p> <p>Effective April 6, 1970, the dose level for the high level animals should be changed to 7.0 g/kg.</p> <p><i>In wk. 17 - beginning with -</i></p>			

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APR 7 1970

CHRONIC TOXICOLOGY SECTION

00003

HAZLETON LABORATORIES PROJECT SHEET

850273

PROJECT SHEET NO. <u>4</u>		PROJECT NO. <u>700-233</u>	
		PROJECT COORDINATOR Jessup/Reno	DATE April 30, 1970
COMPOUND(S) SC-18862	LOT NO(S). A-3427	RECEIPT DATE 10-29-69	L.N.-NUMBER(S) 12,237B
DIVISIONS PARTICIPATING Toxicology	DISTRIBUTION: CENTRAL FILE (2) EACH DIV. PARTICIPATING EACH DIV. DIRECTOR		Sponsor PROJ. COORD. DATA PROCESSING
PHYSICAL AND CHEMICAL PROPERTIES			
SPECIAL PRECAUTIONS (INDICATE PERSONNEL HAZARDS, PROTECTIVE INSTRUCTIONS)			
REFERENCE INFORMATION			
PROGRESS REPORTS DUE	FINAL REPT DUE on completion	INITIALS FER:mtg	SIGNATURE (PHO) COORDINATOR <i>[Signature]</i>
EXPERIMENTAL WORK to be performed in Small Animal Toxicology Section			
<p><u>Two-Year Toxicity Study - Rats</u></p> <p>This project sheet is issued to correct the errors in the Postmortem Procedures from Project Sheet No. 1.</p>			

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MAY 4 1970

CHRONIC TOXICOLOGY SECTION

00007

Project Sheet No. 4
Project No. 700-233

- 2 -

April 30, 1970

POSTMORTEM PROCEDURES

Organ Weights - Indicated in Column I from each animal.

Preservation of Tissues - Indicated in Column II, from each animal, in 10% neutral buffered formalin unless indicated otherwise.

Histopathological Evaluation - From 30 males and 30 females in Groups 1 and 5.

- From 15 males and 15 females in Groups 2, 3, and 4.

--From males and females in Groups .

Tissues	I Wgt.	II Fixed	Histopathology, Group No.						Special Observations or Procedures
			1	2	3	4	5	6	
Brain		X✓							
Pituitary		X✓	X				X		
Spinal Cord		X✓							
Eye		X✓							
Salivary Gland		X✓							
Thyroid	X✓	X✓	X				X		
Parathyroid									
Thymus									
Trachea									
Esophagus									
Lung		X✓							
Heart	X✓	X✓	X				X		
Liver	X✓	X✓	X	X	X	X	X		
EXCLUDED									
Spleen	X✓	X✓	X				X		
Kidney	X✓	X✓	X	X	X	X	X		
Adrenal		X✓	X				X		
Stomach		X✓	X	X	X	X	X		
Pancreas		X✓	X				X		

000008

B = both sexes; M = male only; F = female only

Project Sheet No. 4
Project No. 700-233

- 3 -

April 30, 1970

POSTMORTEM PROCEDURES (Continued)

B = both sexes; M = male only; F = female only

00005

HAZLETON LABORATORIES PROJECT SHEET

850273

PROJECT SHEET NO. <u>5</u>		PROJECT NO. <u>700-233</u>	
		PROJECT COORDINATOR Reno	DATE December 1, 1970
COMPOUND(S) SC-18862	LOT NO(S) 	RECEIPT DATE 10-29-70	LH-NUMBER(S) 12,2378
DIVISIONS PARTICIPATING Toxicology	DISTRIBUTION: CENTRAL FILE (2) EACH DIV. PARTICIPATING EACH DIV. DIRECTOR		Sponsor PROJ. COORD. DATA PROCESSING
PHYSICAL AND CHEMICAL PROPERTIES			
SPECIAL PRECAUTIONS (INDICATE PERSONNEL HAZARDS, PROTECTIVE INSTRUCTIONS)			
REFERENCE INFORMATION			
PROGRESS REPORTS DUE	FINAL REPT DUE on completion	INITIALS FER:lgm	SIGNATURE (PROJ. COORDINATOR) <i>J. S. Reno</i>
EXPERIMENTAL WORK to be performed in Small Animal Toxicology Section			
<p><u>Two-Year Toxicity Study - Rats</u></p> <p><u>Change in Dosage Level</u> - This will document that, effective October 19, 1970 (beginning Week #3 of study), the dose for the high level rats was increased to 8.0 g/kg/day.</p> <p><i>beginning Week 45</i></p>			

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DEC 4 1970

CHRONIC TOXICOLOGY SECTION

00010

Item E

HAZLETON LABORATORIES PROJECT SHEET

850273

PROJECT SHEET NO. <u>6</u>		PROJECT NO. <u>700-233</u>	
		PROJECT COORDINATOR Reno/Trutter	DATE November 4, 1971
COMPOUND(S) SC-18862	LOT NO(S). 76050A	RECEIPT DATE 11/4/71	LH-NUMBER(S) 12,237EB
DIVISIONS PARTICIPATING Toxicology	DISTRIBUTION: CENTRAL FILE (2) EACH DIV. PARTICIPATING EACH DIV. DIRECTOR		Sponsor PROJ. COORD. DATA PROCESSING
PHYSICAL AND CHEMICAL PROPERTIES			
SPECIAL PRECAUTIONS (INDICATE PERSONNEL HAZARDS, PROTECTIVE INSTRUCTIONS)			
REFERENCE INFORMATION			
PROGRESS REPORTS DUE	FINAL REPT DUE on completion	INITIALS FER:lgn	SIGNATURE (PROJ. COORDINATOR) <i>F. E. R. Reno</i>
EXPERIMENTAL WORK to be performed in Small Animal Toxicology Section.			
<p><u>Two-Year Toxicity Study - Rats</u></p> <p>This project sheet is issued to modify Postmortem Procedures as outlined on the following page.</p> <p>This represents an increase in the scope of the histopathology.</p>			

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NOV 8 1971

CHRONIC TOXICOLOGY SECTION

00011

Project Sheet No. 6
Project No. 700-233 (850273)

- 2 -

PATH-TOX. PROJ. NO. 33817
November 4, 1971

PHARMACOLOGIC EFFECTS Evaluation of the following parameters provides evidence of compound absorption:

20) POSTMORTEM PROCEDURES*

TISSUES	A Wt.	B Fix	C (Micro)				
			L	M	H	VH	C
Stomach		X	10	10	40	40	60
Small intestine		X	10	10	40	40	60
Large intestine		X	10	10	40	40	60
Lung		X	10	10	40	40	60
Heart	X	X	10	10	40	40	60
Kidney	X	X	10	10	40	40	60
Liver	X	X	10	10	40	40	60
Gallbladder							
Spleen	<i>Hand</i>	X	10	10	40	40	60
Pancreas		X	10	10	40	40	40
Pituitary		X	10	10	40	40	60
Thyroid	X	X	10	10	40	40	60
Adrenal	<i>Hand</i> X	X	10	10	40	40	60
Gonad	X	X	10	10	40	40	60
Uterus/sem.v.	X	X	10	10	40	40	60
Vagina/prostate	M	X	10	10	40	40	60
Mammary gland <i>R. 4 & 5</i>	<i>Hand</i> X	X	—	—	40	40	60
Brain; 2 levels		X	—	—	—	40	60
Spinal cord		X	—	—	—	40	60
Nerve with muscle		X	—	—	—	40	60
Eye, R. & L.	<i>Hand</i> X	X	—	—	—	40	60
Urinary bladder	<i>Hand</i> X	X	40	40	40	40	60
Salivary gland		X	—	—	40	40	60
Lymph node <i>Hand</i>		X	—	—	40	40	60
Thymus			—	—	—	—	—
Bone marrow <i>Hand</i>		X	—	—	—	30	30
Rib junction		X	—	—	—	—	—
Skin		X	—	—	—	—	—
Unusual lesions		X	40	40	40	60	60
Unusual lesions		X	40	40	40	60	60

- A — The organs weighed from each animal.
B — The tissues preserved from each animal.
C — Tissues examined microscopically from the indicated no. of animals of each sex.

Additional postmortem procedures:

- * All animals, survivors or non-survivors, in the control, high & very high dose levels shall be examined as indicated. The indicated number of survivors at the remaining dose levels shall be selected at random and examined similarly. Usual and unusual lesions from all animals shall be examined microscopically.

(Examine 10 Males each, C & VH.)

00012

Item F

HAZLETON LABORATORIES PROJECT SHEET

PROJECT SHEET NO. <u>7</u>		PROJECT NO. <u>700-233</u>	
		PROJECT COORDINATOR <u>Reno</u>	DATE <u>May 1, 1972</u>
COMPOUND(S) <u>SC-18862</u>	LOT NO(S)	RECEIPT DATE	LH-NUMBER(S)
DIVISIONS PARTICIPATING <u>Toxicology</u>	DISTRIBUTION: CENTRAL FILE (2) EACH DIV. PARTICIPATING EACH DIV. DIRECTOR		Sponsor PROJ. COORD. DATA PROCESSING
PHYSICAL AND CHEMICAL PROPERTIES			
SPECIAL PRECAUTIONS (INDICATE PERSONNEL HAZARDS, PROTECTIVE INSTRUCTIONS)			
REFERENCE INFORMATION <u>Telephone conversation with Dr. R. G. McConnell</u>			
PROGRESS REPORTS DUE	FINAL REPT DUE on compl .	INITIALS FER:da	SIGNATURE (PROJ. COORDINATOR) <u>[Signature]</u>
EXPERIMENTAL WORK to be performed in Histology and Pathology Section			
<u>Two-Year Toxicity Study - Rats</u>			
<u>Histopathological Examination of Urinary Bladder</u>			
<p>Four sections are to be examined from each rat instead of the one previously indicated. With urinary bladder cut in half longitudinally, both hemispheres are to be imbedded and two longitudinal sections cut from each hemisphere. There should be approximately 50 microns between each section.</p> <p>This procedure should be done on all rats (total approximately 1760 tissues).</p>			
<p>RECEIVED</p> <p>MAY 3 1972</p> <p>CHRONIC TOXICOLOGY SECTION</p> <p>00013</p>			

Item G

HAZLETON LABORATORIES PROJECT SHEET

PROJECT SHEET NO. <u>8</u>		PROJECT NO. <u>700-233</u>	
		PROJECT COORDINATOR <u>Reno/Trutter</u>	DATE <u>April 30, 1973</u>
COMPOUND(S) <u>SC-18862</u>		LOT NO(S)	RECEIPT DATE LH-NUMBER(S)
DIVISIONS PARTICIPATING <u>Toxicology</u>		DISTRIBUTION: CENTRAL FILE (2) EACH DIV. PARTICIPATING EACH DIV. DIRECTOR Sponsor PROJ. COORD. DATA PROCESSING	
PHYSICAL AND CHEMICAL PROPERTIES			
SPECIAL PRECAUTIONS (INDICATE PERSONNEL HAZARDS, PROTECTIVE INSTRUCTIONS)			
REFERENCE INFORMATION			
PROGRESS REPORTS DUE	FINAL REPT DUE on compl.	INITIALS FER:da	SIGNATURE (PROJ. COORDINATOR)
EXPERIMENTAL WORK to be performed in Histopathology This project is being reopened at the request of the sponsor. <u>Histopathological Evaluation of Tissues</u> Two sections of brain from all animals in Groups No. 2, No. 3, and No. 4 are to be sectioned, stained with H&E, and examined microscopically.			

00014

Item H

December 7, 1971

MEMO TO: Dr. Sammeta (Biostatstician)
Dr. F. Saunders (Biol. Res. Adviser)
Dr. Ranney (Metab. Represent.)
Dr. Polk (Clinical Represent.)
Dr. McConnell (P-T Dept. adviser)
Dr. Hutsell (Bio-Anal. Lab)

COPY TO: Dr. Reno (Hazleton Labs.)

FROM: Dr. Rao

SUBJECT: SC-18862: 104 week oral toxicity study in the rat.
P-T No. 838H71; Protocol amendment No. 2. Clinical
Laboratory Measurements.

Please analyze serum phenylalanine (PA) of 5 males and 5 females from the control, high and very high dose groups of the above study at termination. Bioanalytical Lab. will receive one ml. of serum specimen (frozen) from Hazleton Laboratories for PA analysis and promptly return the data to toxicology section. Immediately thereafter this data will be forwarded to Hazleton Laboratories for inclusion in their report.

K. S. Rao.
K. S. Rao

KSR:ml

5101

Item I

October 7, 1971

MEMO TO: Dr. Moe
Dr. F. Saunders
Dr. Polk
Dr. Sammeta
Dr. Ranney

COPY TO: Dr. McConnell

FROM: Dr. Rao

SUBJECT: SC-18862; updating sweetener file; P.T. No. 838H71
protocol revised.

. Please find enclosed, a revised complete protocol of the two year study in the rat with SC-18862 (P.T. No. 838H71). As the latest protocols and status reports are shipped to you, please update your files by discarding the old ones.

K. S. Rao.

K. S. Rao

KSR:ml
enclosure

FINAL PROTOCOL FOR A PRECLINICAL SAFETY STUDY OF SC-18862

COMMERCIAL LAB PROJ. NO. 700-233 AMENDED (1)(2) 3 4 5 PATH-TOX PROJ. NO. 838H71

- 1) Protocol finalized _____ Treatment initiated 12.11.69 Animals terminated 12.11.71 Final report 010^{dst} fir
- Cpd. needed (kg): Total 427 First 4 wks. _____ Ordered _____ Del'vy _____ cst fir
- 3) Study title & objectives:
SC-18862: 104 week oral toxicity study in the rat. P-T No. 838H71. Evaluation of the toxicologic and tumorigenic potential in the rat.
- 4) Species, strain, sex, (M,F): Rat; CRcd; M, F. Age (wk) at Rx start: 4
- 5) Rx duration (wks): 104 Route & Freq. of admin.: Oral; continuous, ad lib.
- 6) Mode of admin.: Mixed with diet.
- 7) Drug-vehicle mixture stability analysis; Rx wks.: Uncertain.
- 8) Est. daily human (50 kg.) dose & route: 1 gm. orally (0.02 gm/Kg) in divided doses.
- 9) Dose levels (gpk daily): Control 0; Low 1; Med. 2; High 4; V.High 6*
- 10) Multiple of human dose: 0; 50; 100; 200; 300
- 11) No. & sex of animals/level: 60 M; 40 M; 40 M; 40 M 40 M
60 F; 40 F; 40 F; 40 F 40 F
*After 8 weeks of treatment dose was increased to 7 g/Kg.
- 12) Total animals required: 440
- 13) Housing & basal diet: Individual; Rockland Rat/Mouse. Complete diet; Powdered.
- 14) General observations (frequency; wks)
Morbidity-mortality: Observe daily and record
Motor & behavioral activity: Daily; summarize weekly.
Body weight: Pre-Rx and weekly thereafter.
Food consumption & dose adjust.: Carefully estimate weekly.
Additional observations: If any record.
- 15) Physical examination (frequency; wks)
Gen'l external features, incl. body orifices & excrement: Pre-Rx and weekly.
Limited neurological: --- Detailed neurological: ---
Ophthalmoscopic: Pre-Rx, 52 weeks and terminally.
Digital palpation for protruding tissue masses: Observe weekly and record.
Body temperature (rectal): ---
Blood pressure and/or ECG: ---

KSR
10/6/71

Revised
checked
10-6-71
KSR

Page 2

PROTOCOL FOR A PRECLINICAL SAFETY STUDY OF SC-18862

16-18:

CLINICAL LABORATORY PROCEDURES*

PATH-TOX. PROJ. NO. 833H71

6163

Specimen collection: individual
(5 /sex/level)

Blood: Collected from tail vein.

Urine: Collected from metabolic cages.

16. HEMATOLOGY

Parameter	No./sex/ level	Rx interval (wks)
Hematocrit.....	5	13,26,52,104
Hemoglobin.....	5	13,26,52,104
Total RBC.....	5	13,26,52,104
Total WBC.....	5	13,26,52,104
Differential.....	5	13,26,52,104
Reticulocyte.....		
Platelets.....		
Coagulation (L-W).		
Pro. time.....		
Activ. PTT.....		
arrow smear.....		
.....		
.....		

17. URINALYSIS

Parameter	No./sex/ level	Rx interval (wks)
Sp. gravity.....	5	13,26,52,104
Bili-Labstix.....		
pH, Bilirubin, Protein, Sugar, Ketones, Blood.	5	13,26,52,104
Urobilinogen.....		
Microscopic.....	5	13,26,52,104
Phenylketones....	5	Each month throughout the study.

18. CLINICAL CHEMISTRY

Parameter	No./sex/ level	Rx interval (wks)	Parameter	No./sex/ level	Rx interval (wks)
BUN.....	5	13,26,52,104	GPT.....	5	13,26,52,104
Uric acid.....			GOT.....	5	104
Glucose.....	5	13,26,52,104	AP.....	5	13,26,52,104
Sodium.....	5	104	BSP.....		
Potassium.....	5	104	Bilirubin.....	5	13,26,52,104
Calcium.....	5	104	OCT.....	5	104
Carbon Dioxide....	5	104	CPK.....		
Total Serum Protein	5	13,26,52,104	Serum Electro-....	5	13,26,52,104
Serum Albumin	5	104	phoresis		
			Serum Chloride....	5	104

* Report actual pre-Rx specimen collection(s) as negative number (wks). Clin. lab
workup done preferably on those animals receiving complete postmortem workup.

- 19) PHARMACOLOGIC EFFECTS Evaluation of the following parameters provides evidence of compound absorption:

2) POSTMORTEM PROCEDURES*

TISSUES	A Wt.	B Fix	C (Micro)				
			L	M	H	VH	C
Stomach		X	10	10	40	40	60
Small intestine		X	10	10	40	40	60
Large intestine		X	10	10	40	40	60
Lung		X	10	10	40	40	60
Heart	X	X	10	10	40	40	60
Kidney	X	X	10	10	40	40	60
Liver	X	X	10	10	40	40	60
Gall bladder							
Spleen		X	10	10	40	40	60
Pancreas		X	10	10	40	40	40
Pituitary		X	10	10	40	40	60
Thyroid	X	X	10	10	40	40	60
Adrenal	X	X	10	10	40	40	60
Gonad	X	X	10	10	40	40	60
Uterus/sem.v.	X	X	10	10	40	40	60
Vagina/prostate	M	X	10	10	40	40	60
Mammary gland ^{R. 4 & 5}	F	X	—	—	40	40	60
Brain; 2 levels		X	—	—	—	40	60
Spinal cord		X	—	—	—	40	60
Nerve with muscle		X	—	—	—	40	60
Eye, R.		X	—	—	—	40	60
Urinary bladder		X	40	40	40	40	60
Salivary gland		X	—	—	40	40	60
Lymph node		X	—	—	40	40	60
Thymus			—	—	—	—	—
Bone marrow		X	—	—	—	30	30
Rib junction		X	—	—	—	—	—
Skin		X	—	—	—	—	—
Unusual lesions		X	40	40	40	60	60
Usual lesions		X	40	40	40	60	60

A -- The organs weighed from each animal.

B -- The tissues preserved from each animal.

C -- Tissues examined microscopically from the indicated no. of animals of each sex.

Additional postmortem procedures:

* All animals, survivors or non-survivors, in the control, high, & very high dose levels shall be examined as indicated. The indicated number of survivors at the remaining dose levels shall be selected at random and examined similarly. Usual and unusual lesions from all animals shall be examined microscopically.

(Examine 10 ^{2/3} each, C & VH.)

21) STATISTICAL EVALUATION OF DATA: PROCEDURES USED

- a) Body wt. change; food & drug consumption:
Group mean \pm S.E.; Appropriate analysis of intergroup variance at each time interval.
- b) Clinical laboratory values:
Group mean \pm S.E.; Appropriate analysis of intergroup variance at each time interval.
- c) Incidence and onset of neoplasms:
Mean incidence and appropriate analysis of intergroup variance at termination.
- d) Randomization procedures:
Simple randomization.

22) INTERIM AND FINAL STUDY REPORTS

The sponsor (Director; Path-Tox Dept) requires a brief quarterly report relating statistically significant changes in items 19a & b, with a general statement on items 14, 15, and 19c, by or on the 1st of Jan, April, July, and October; serious adverse findings are to be reported immediately.

Protocol Distribution List

Design Committee Members:

- | | |
|---------------------------|-------------------------|
| 1) <u>Dr. Sammeta</u> | (Biostatistician) |
| 2) <u>Dr. F. Saunders</u> | (Biol. Res. Asst. Dir.) |
| 3) <u>Dr. Ranney</u> | (Drug Metab. Rep.) |
| 4) <u>Dr. Polk</u> | (Clinical represent.) |
| 5) <u>Dr. Rao</u> | (P-T Dept. monitor) |
| 6) <u>Dr. McConnell</u> | (P-T Dept. adviser) |

Technical Staff

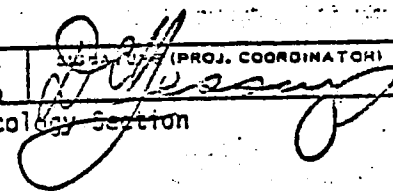
- | | |
|----------|------------------|
| 1) _____ | (Path. Lab) |
| 2) _____ | (Autopsy Lab) |
| 3) _____ | (Bio-Anal. Lab) |
| 4) _____ | (Gen'l Tox. Lab) |
| 5) _____ | (Hematology Lab) |
| 6) _____ | (Pathologist) |

Item A'

THIS PROJECT SHEET REPLACES ALL PREVIOUS SHEETS. PLEASE DESTROY PREVIOUS PROJECT SHEETS NO. 1 AND NO. 2.

HAZLETON LABORATORIES PROJECT SHEET

850273

PROJECT SHEET NO. <u>1</u>		PROJECT NO. <u>700-233</u>																													
PROJECT COORDINATOR Jessup/Reno		DATE December 4, 1969																													
COMPOUND(S) SC-18862 ; P-T No. 838H71	LOT NO(S). A-3427	RECEIPT DATE 10-29-69	LH-NUMBER(S) 12,2378																												
DIVISIONS PARTICIPATING Toxicology	DISTRIBUTION: CENTRAL FILE (2) EACH DIV. PARTICIPATING EACH DIV. DIRECTOR																														
PRJ. COORD. DATA PROCESSING																															
PHYSICAL AND CHEMICAL PROPERTIES																															
SPECIAL PRECAUTIONS (INDICATE PERSONNEL HAZARDS, PROTECTIVE INSTRUCTIONS)																															
REFERENCE INFORMATION																															
PROGRESS REPORTS DUE	FINAL REPT DUE on completion	INITIALS DCI:sfh	SIGNATURE (PROJ. COORDINATOR) 																												
EXPERIMENTAL WORK to be performed in Small Animal Toxicology Section																															
<p><u>Two-Year Toxicity Study - Rats</u></p> <p>Animal Groups - Four hundred ^{forty} weanling albino rats, ²²⁰ males and ²²⁰ females, will be selected at random and placed into the following groups:</p> <table border="1"> <thead> <tr> <th>Group No.</th> <th colspan="2">No. of Animals</th> <th>Dietary Levels</th> </tr> <tr> <th></th> <th>male</th> <th>female</th> <th>g/kg</th> </tr> </thead> <tbody> <tr> <td>1 (Control)</td> <td>60</td> <td>60</td> <td>0</td> </tr> <tr> <td>2</td> <td>40</td> <td>40</td> <td>1</td> </tr> <tr> <td>3</td> <td>40</td> <td>40</td> <td>2</td> </tr> <tr> <td>4</td> <td>40</td> <td>40</td> <td>4</td> </tr> <tr> <td>5</td> <td>40</td> <td>40</td> <td>6</td> </tr> </tbody> </table> <p>Group No. 1 will serve as a control group and will be treated in the same manner as the other groups except that no test material will be administered. Group No. 5 will receive 6 g/kg during the first two months. The dose will be increased probably after two months, and the changed dose level will be indicated by the Project Manager at that time.</p> <p>The rats in each group will be individually housed.</p>				Group No.	No. of Animals		Dietary Levels		male	female	g/kg	1 (Control)	60	60	0	2	40	40	1	3	40	40	2	4	40	40	4	5	40	40	6
Group No.	No. of Animals		Dietary Levels																												
	male	female	g/kg																												
1 (Control)	60	60	0																												
2	40	40	1																												
3	40	40	2																												
4	40	40	4																												
5	40	40	6																												

Project Sheet No. 1
Project No. 700-233 (850273)

- 2 -

December 4, 1969

Water and the appropriate diets will be freely available during the course of the study.

Diet and Compound Administration - In order to ensure proper dosing on a g/kg body weight basis during the weeks of rapid growth, the following regimen will be followed:

- Weeks 0 through 4 - Calculate individual food consumption three times per week, and adjust dose according to body weight change.
- Weeks 5 through 13 - Calculate individual food consumption two times per week, and adjust dose according to body weight change.
- Weeks 14 through 26 - Calculate individual food consumption every week, and adjust dose according to body weight change.
- Weeks 27 through 52 - Calculate individual food consumption bi-weekly, and adjust dose according to body weight change.
- Weeks 53 to termination - Calculate food consumption monthly, and adjust dose according to body weight change.

NOTE: In order to conserve test material during the stage of rapid growth do not discard remaining feed every two days or three days, but adjust concentration by addition of diet or test material where appropriate.

Observations - Individual body weights and food consumption will be recorded as indicated above.

Observations of gross signs of toxicity; pharmacological effects; and the incidence, size, and location of tumors will be recorded at the same intervals.

The rats will be observed daily for mortality.

Necropsies will be performed on all rats which die during the course of the study, and tissues will be taken.

Ophthalmoscopic Examination - Will be performed on all animals initially, at one year, and at termination.

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December 4, 1969

Clinical Studies - The following clinical studies will be performed on five male and five female animals from the control and each test group:

Hematology - At 13, 26, 52, and 104 weeks:

hematocrit	erythrocyte count	differential leukocyte
hemoglobin	total leukocyte count	count

Clinical Biochemistry - At 13, 26, and 52 weeks:

fasting blood sugar	total serum bilirubin	serum alkaline phos-
blood urea nitrogen	serum glutamic-pyruvic	phatase
total serum protein	transaminase	serum electrophoresis

- At 104 weeks:

fasting blood sugar	serum potassium	serum alkaline phos-
blood urea nitrogen	serum chloride	phatase
total serum protein	carbon dioxide	serum glutamic-oxaloacetic
total serum bilirubin	serum calcium	transaminase
serum albumin	serum glutamic-pyruvic	serum electrophoresis
serum sodium	transaminase	

Urine Analysis - At 13, 26, 52, and 104 weeks (pooled samples):

pH	ketones	microscopic examination
specific gravity	total protein	of sediment
glucose	bilirubin	

- At monthly intervals throughout the study:

phenylketonuria (dipstick method)

Terminal Necropsy - At 104 weeks the study will be terminated, and the following procedures will be followed:

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POSTMORTEM PROCEDURES

Organ Weights - Indicated in Column I from each animal at terminal sacrifice.

Preservation of Tissues - Indicated in Column II, from each animal, in 10% neutral buffered formalin unless indicated otherwise.

Histopathological Evaluation - From 30 males and 30 females in Groups 1 and 4.

- From 15 males and 15 females in Groups 2 and 3.

- From males and females in Groups .

Tissues	I Wgt.	II Fixed	Histopathology, Group No.						Special Observations or Procedures
			1	2	3	4	5	6	
Brain		X							
Pituitary		X	X			X			
Spinal Cord		X							
Eye		X							
Salivary Gland		X							
Thyroid	X	X	X			X			
Parathyroid									
Thymus									
Trachea									
Esophagus									
Lung		X							
Heart	X	X	X			X			
Liver	X	X	X	X	X	X			
Gallbladder									
Spleen	X	X	X			X			
Kidneys	X	X	X	X	X	X			
Adrenals		X	X			X			
Stomach		X	X	X	X	X			
Pancreas		X	X			X			

B = both sexes; M = male only; F = female only

December 4, 1969

Special Observations
or Procedures

B = both sexes; M = male only; F = female only

December 4, 1969

Analysis and Report - At termination of the study, the results will be reported in full giving:

experimental design
general physical appearance
behavior
effects on body weight, food
consumption, and survival

gross signs of toxic or
pharmacological effects
clinical findings
individual gross and microscopic
necropsy findings

- Statistical evaluation:

body weights
food consumption
survival

organ weights
organ/body weight ratios

- Tables will be furnished showing:

mean weekly body weights
weight ranges
food consumption
survival data
individual hematological values
individual biochemical values

results of urine analysis
mean terminal body weights,
organ weights, and organ/body
weight ratios
tissue mass incidence

Mean weekly body weights and food and compound consumption will be presented graphically.

HAZLETON LABORATORIES PROJECT SHEET

0173
850273

PROJECT SHEET NO. <u>3</u>		PROJECT NO. <u>700-233</u>	
		PROJECT COORDINATOR <u>Jessup/Reno</u>	DATE <u>April 6, 1970</u>
COMPOUND(S) <u>SC-18862</u>	LOT NO(S). <u>A-3427</u>	RECEIPT DATE <u>10-29-69</u>	LH-NUMBER(S) <u>12,2378</u>
DIVISIONS PARTICIPATING <u>Toxicology</u>	DISTRIBUTION: CENTRAL FILE (2) EACH DIV. PARTICIPATING EACH DIV. DIRECTOR <input checked="" type="checkbox"/> Sponsor PROJ. COORD. DATA PROCESSING		
PHYSICAL AND CHEMICAL PROPERTIES			
SPECIAL PRECAUTIONS (INDICATE PERSONNEL HAZARDS, PROTECTIVE INSTRUCTIONS)			
REFERENCE INFORMATION			
PROGRESS REPORTS DUE	FINAL REPT DUE <u>on completion</u>	INITIALS <u>FER:mtg</u>	SIGNATURE PROJ. COORDINATOR <u>[Signature]</u>
EXPERIMENTAL WORK to be performed in Small Animal Toxicology			
<u>Two-Year Toxicity Study - Rats</u> Effective April 6, 1970, the dose level for the high level animals should be changed to 7.0 g/kg.			

Item C'

0170

HAZLETON LABORATORIES PROJECT SHEET

850273

PROJECT SHEET NO. <u>4</u>		PROJECT NO. <u>700-233</u>	
		PROJECT COORDINATOR Jessup/Reno	DATE April 30, 1970
COMPOUND(S) SC-18862	LOT NO(S). A-3427	RECEIPT DATE 10-29-69	LM-NUMBER(S) 12,2378
DIVISIONS PARTICIPATING Toxicology	DISTRIBUTION: CENTRAL FILE (2) EACH DIV. PARTICIPATING EACH DIV. DIRECTOR		
Sponsor <input checked="" type="checkbox"/> PROJ. COORD. DATA PROCESSING			
PHYSICAL AND CHEMICAL PROPERTIES			
SPECIAL PRECAUTIONS (INDICATE PERSONNEL HAZARDS, PROTECTIVE INSTRUCTIONS)			
REFERENCE INFORMATION			
PROGRESS REPORTS DUE	FINAL REPT DUE on completion	INITIALS FER:mtc	SIGNATURE OF PROJ. COORDINATOR <i>[Signature]</i>
EXPERIMENTAL WORK to be performed in Small Animal Toxicology Section			
<u>Two-Year Toxicity Study - Rats</u> This project sheet is issued to correct the errors in the Postmortem Procedures from Project Sheet No. 1.			

Project Sheet No. 4
Project No. 700-233

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6100
April 30, 1970

POSTMORTEM PROCEDURES

Organ Weights - Indicated in Column I from each animal.

Preservation of Tissues - Indicated in Column II, from each animal, in 10% neutral buffered formalin unless indicated otherwise.

Histopathological Evaluation - From 30 males and 30 females in Groups 1 and 5.

- From 15 males and 15 females in Groups 2, 3, and 4.

- From males and females in Groups .

Tissues	I Wgt.	II Fixed	Histopathology, Group No.						Special Observations or Procedures
			1	2	3	4	5	6	
Brain		X							
Pituitary		X	X				X		
Spinal Cord		X							
Eye		X							
Salivary Gland		X							
Thyroid	X	X	X				X		
Parathyroid									
Thymus									
Trachea									
Esophagus									
Lung		X							
Heart	X	X	X				X		
Liver	X	X	X	X	X	X	X		
ESOPHAGUS									
Spleen	X	X	X				X		
Kidney	X	X	X	X	X	X	X		
Adrenal		X	X				X		
Stomach		X	X	X	X	X	X		
Pancreas		X	X				X		

B = both sexes; M = male only; F = female only

HAZLETON LABORATORIES PROJECT SHEET

850273-43

PROJECT SHEET NO. <u>6</u> <u>PT 838471</u>		PROJECT NO. <u>700-233</u>	
		PROJECT COORDINATOR <u>Reno/Trutter</u>	DATE <u>November 4, 1971</u>
COMPOUND(S) <u>SC-18962</u>	LOT NO(S). <u>76050A</u>	RECEIPT DATE <u>11/4/71</u>	LN-NUMBER(S) <u>12,237B5</u>
DIVISIONS PARTICIPATING <u>Toxicology</u>	DISTRIBUTION: CENTRAL FILE (2) EACH DIV. PARTICIPATING EACH DIV. DIRECTOR.		Sponsor <input checked="" type="checkbox"/> PROJ. COORD. DATA PROCESSING
PHYSICAL AND CHEMICAL PROPERTIES			
SPECIAL PRECAUTIONS (INDICATE PERSONNEL HAZARDS, PROTECTIVE INSTRUCTIONS)			
REFERENCE INFORMATION			
PROGRESS REPORTS DUE	FINAL REPT DUE on completion	INITIALS <u>FER:lgm</u>	SIGNATURE (PROJ. COORDINATOR) <u>[Signature]</u>
EXPERIMENTAL WORK to be performed in Small Animal Toxicology Section			
<p><u>Two-Year Toxicity Study - Rats</u></p> <p>This project sheet is issued to modify Postmortem Procedures as outlined on the following page.</p> <p>This represents an increase in the scope of the histopathology.</p>			

PROTOCOL FOR A PRECLINICAL SAFETY STUDY OF SC-18862

Project Sheet No. 6
Project No. 700-233 (850273)

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PATH-TOX. PROJ. NO. 333871
November 4, 1971

PHARMACOLOGIC EFFECTS Evaluation of the following parameters provides evidence of compound absorption:

20) POSTMORTEM PROCEDURES*

TISSUES	A Wt.	B Fix	C (Micro)				
			L	M	H	VH	C
Stomach		X	10	10	40	40	60
Small intestine		X	10	10	40	40	60
Large intestine		X	10	10	40	40	60
Lung		X	10	10	40	40	60
Heart	X	X	10	10	40	40	60
Kidney	X	X	10	10	40	40	60
Liver	X	X	10	10	40	40	60
Gonadoblastoma							
Spleen	0	X	10	10	40	40	60
Pancreas		X	10	10	40	40	40
Pituitary		X	10	10	40	40	60
Thyroid	X	X	10	10	40	40	60
Adrenal	X	X	10	10	40	40	60
Gonad	X	X	10	10	40	40	60
Uterus/sem.v.	X	X	10	10	40	40	60
Vagina/prostate	M	X	10	10	40	40	60
Mammary gland R. 4 & 5	F	X	—	—	40	40	60
Brain; 2 levels		X	—	—	—	40	60
Spinal cord		X	—	—	—	40	60
Nerve with muscle		X	—	—	—	40	60
Eye, R.		X	—	—	—	40	60
Urinary bladder		X	40	40	40	40	60
Salivary gland		X	—	—	40	40	60
Lymph node		X	—	—	40	40	60
Thymus			—	—	—	—	—
Bone marrow		X	—	—	—	30	30
Rib junction		X	—	—	—	—	—
Skin		X	—	—	—	—	—
Unusual lesions		X	40	40	40	30	60
Usual lesions		X	40	40	40	60	60

A -- The organs weighed from each animal.

B -- The tissues preserved from each animal.

C -- Tissues examined microscopically from the indicated no. of animals of each sex.

Additional postmortem procedures:

* All animals, survivors or non-survivors, in the control, high & very high dose levels shall be examined as indicated. The indicated number of survivors at the remaining dose levels shall be selected at random and examined similarly. Usual and unusual lesions from all animals shall be examined microscopically.

(Examine 10 Males each, C & VH.)

APPENDIX IV-2

RESUME OF HAZLETON PROJECT SHEETS, SEARLE PROTOCOL,
AND AMENDMENTS

The following comments arranged in chronological sequence, point out some of the inconsistencies and problems noted by UAREP in their attempt to review and reconstruct the evolution of the protocol and their amendments with respect to the work as actually reported in E-33,34. No attempt is made to repeat many details that were carried out as reflected in the report submitted to FDA. Copies of the documents discussed are in Appendix IV-1. Both Hazleton and Searle supplied copies of the basic Hazleton Project Sheets 1 and 2, dated December 4, 1969, and there were minor differences in notations from the two sources.

Hazleton Project Sheet 1 & 2 - The front page says, "THIS PROJECT SHEET REPLACES ALL PREVIOUS SHEETS. PLEASE DESTROY PREVIOUS PROJECT SHEETS NO. 1 AND NO. 2, Project Sheet No. 1. Date: December 4, 1969." All other five project pages (pages 2-6) say "Project Sheet No. 1. December 4, 1969." The underlining in the above quotation was on the copy obtained from Searle, but not on the copy from Hazleton. UAREP doesn't know the date this front sheet was typed, and whether it represents the third version of the Hazleton Project (which it would be if number 1 and number 2 were destroyed), and whether some sheets such as pages 4 and 5 were uncorrected and simply duplicated from earlier versions of the protocol. The fact that the Hazleton copy was time-stamped December 8,

1969, and that all pages are dated December 4, 1969, would favor their all being typed at the same time. Earlier versions of the Project Sheets were not available to UAREP upon request. The copy of page 1 received from HLA indicates 420 animals required, but the copy received from Searle has this crossed out and the correct figure of 440 written in. This could represent an error in addition because the correct number of animals per group is given on the same page.

Page 2 - The schedule for aspartame consumption given on page 2 of the project sheets was generally followed with minor variations shown in Appendix IV-4.

Page 2 indicates that food should be conserved during rapid growth phase by not discarding remaining feed every two or three days, but by adjusting the concentration by addition of diet or test material when appropriate. The Entry Book report does not state that this procedure was followed. When queried by UAREP regarding the procedure, Dr. Reno replied two months later, ". . . dose levels were adjusted on an mg/kg/day basis three times weekly during weeks one through four, and twice weekly in weeks five through thirteen. In order to reduce the amount of test material wasted, old feed was removed and weighed, then additional feed or compound was added to the uneaten feed as necessary to achieve the new dietary level. This new diet was then presented to the animals. This procedure was followed only for the first thirteen weeks of the study. Only in this instance was new feed added to old feed." This procedure would have made it very difficult

to compute the actual amount of compound consumption by the animal.

Ophthalmoscopic examination was requested at the initiation, at one year, and at termination of experiment. Initial eye examinations were performed on 12-12-69, two days prior to the start of the experiment, and at 42 weeks, 52 weeks, and 104 weeks. UAREP has seen no documentation of a request for examination at 42 weeks. UAREP was provided no raw data relative to the monthly testing for phenylketonuria as mentioned on page 3 of the project sheets, but on page 41 of the Entry Book it is indicated that the results were negative except in three instances.

Page 4 - This page, relating to histopathology dated December 4, 1969, specifies that tissues from 30 males and 30 females in Groups 1 and 4, and 15 males and 15 females in Groups 2 and 3 were to be evaluated microscopically. The attached page 1 of the same document indicates that there were to be five groups of animals in this experiment, with 60 males and 60 females in the control group and 40 males and 40 females in each of the four experimental groups. This discrepancy could be explained by an updating in experiment design shown on the front page, but not on page four of the project sheets. However, all pages are dated December 4 and the front page was time-stamped December 8. On the copy of the project sheet submitted by Hazleton, but not shown on pages 4 and 5 of the copy obtained by Searle, a number of handwritten "X's" have been placed in the column specifying the tissues to be weighed at the time of necropsy such as adrenals, prostate, seminal vesicle, and uterus.

HLA Project Sheet No. 3 - This memo dated 4-6-70 increased the Group 5 dosage from 6 to 7 g/kg body weight/day effective 4-6-70. There was no corresponding Searle protocol amendment.

HLA Project Sheet No. 4 - April 30, 1970 - Errors in postmortem procedures are corrected from Project Sheet 1 to the extent of recognizing the existence of five groups of animals and requesting histopathological evaluation on 30 males and 30 females in Groups 1 and 5 and 15 males and 15 females in Groups 2, 3, and 4. The organs which had the handwritten "X" specifying "to be weighed" on the initial protocol sheet (adrenal, prostate, seminal vesicle and uterus), now have handwritten question marks. A question mark also appears adjacent to urinary bladder.

HLA Project Sheet No. 5 - This memo dated December 1, 1970 and date-stamped December 4, 1970, increased the dosage in Group 5 males and females from 7 to 8 g/kg body weight/day, effective October 19, 1970, which was six weeks prior to the time this memo was dated. On the memo "beginning week 42 of study" has been changed to week 45. UAREP's review of the records indicated the change was made at week 44.

Searle Amended Protocol No. 2 - Earlier Searle protocols were indicated not to be available. However, this document initialed by Drs. McConnell and Rao on October 6, 1971 and transmitted by memo dated October 7, presents the existing status approximately 22 months after initiation of the experiments. The specifications of this amended protocol were followed as indicated in the results summarized in the Entry Book submitted to FDA with exceptions as noted in this report. The clinical laboratory determinations were made on five animals per sex per level

for hematology, urinalysis, and clinical chemistry parameters as shown in Appendix IV-1 at the prescribed intervals. Page 4, section 22 of the protocol specifies, "INTERIM AND FINAL STUDY REPORTS. The sponsor (Director; Path-Tox Dept) requires a brief quarterly report relating statistically significant changes in items 19 a & b, with a general statement on items 14, 15, and 19c, by or on the first of Jan, April, July, and October; serious adverse findings are to be reported immediately." Sections 14 and 15 of the protocol relate to general observations and physical examinations on the animals whereas section 19 is entitled "PHARMACOLOGIC EFFECTS. Evaluation of the following parameters provides evidence of compound absorption:" There are no entries or subsections a, b, or c under 19. UAREP is unable to precisely evaluate the information requested under the important parameter of evidence of compound absorption. However, UAREP's request to Searle and Hazleton to see copies of the specified quarterly reports produced only an explanation from Hazleton that they were sent to Searle.

HLA Project Sheet No. 6 - November 4, 1971 - The postmortem procedures are again modified, this time by making a page of the Searle protocol a part of the Hazleton Project Sheets. Thus, it appears that 23 months after beginning a two year experiment, the Searle and Hazleton protocols coincide on the postmortem procedures and histopathologic observations to be made. The "X" for weighing spleens has been blotted out and the "X" for weighing adrenals hand written. Although there are only 40 animals in the very high dosage group, the protocol sheet requests that 60 have all unusual lesions as well as usual lesions sectioned.

Searle Protocol Amendment No. 2 (12-7-71) - This is a request for analysis at termination of serum L-phenylalanine on five males and five females from control, high, and very high groups. No supporting project sheet from Hazleton noting these changes was supplied UAREP although the change is indicated in the HLA internal memo dated 12-3-71 from Dr. Reno/Trutter to Mike Elliott. E-33, Appendix Table 3, page 68, gives the results of the L-phenylalanine determinations.

Hazleton Protocol Sheet No. 7 (5-1-72) - Referring to a telephone conversation with Dr. R. G. McConnell, it was requested that four sections of urinary bladder of all rats be evaluated microscopically (total approximately 1760 tissues).

HLA Project Sheet No. 8 (4-30-73) - Sponsor requested that project be reopened, approximately two years after the termination date of the experiment. Two brain sections from all rats in Groups 2, 3, and 4 were to be stained with H & E and examined microscopically. Hazleton Project Sheet 8 was not supported by Searle protocol amendments, and a signature of the Project Coordinator did not appear on the sheet.

APPENDIX IV-3
SUMMARY OF VARIATIONS AS REPORTED IN CLINICAL OBSERVATIONS MADE FOR
TISSUE MASSES AND NODULES FOR E-33,34

<u>Group</u>	<u>Animal Number</u>	<u>Observation</u>
1F	83666	Swelling reported week 64--right and left inguinal Not reported week 72
1F	83668	Nodule reported week 92--right axilla Not reported week 96 Reported week 104--chest
1F	83674	Nodule reported week 64--anus Not reported week 68 Swelling reported week 76--chest Not reported week 80 Reported as nodule week 100--right axilla
1F	83677	Nodule reported week 60--right inguinal Not reported week 84 Reported as swelling week 92--right hind leg
1F	83680	Swelling reported week 60--right and left inguinal Not reported week 64
1F	83684	Nodule reported week 80--right inguinal Not reported week 88
1F	83689	Swelling reported week 50--mouth Not reported week 52
1F	83690	Swelling reported week 76--lower midline Not reported week 80
1F	83694	Nodule reported week 72--right and left inguinal Not reported week 84
1F	83702	Nodule reported week 68--right axilla Not reported week 72
1F	83705	Nodule reported week 84--anus Not reported week 88
1F	83708	Nodule reported week 84--left inguinal Not reported week 88
1F	83713	Nodule reported week 64--right and left inguinal Not reported week 84 Nodule reported week 68--right axilla Not reported week 80 Reported week 92--left axilla
1F	83723	Nodule reported week 76--left inguinal Not reported week 88 Reported week 104--left inguinal, anus
1F	83724	Nodule reported week 76--left inguinal Not reported week 100 Nodule reported week 84--left axilla Not reported week 92
2M	83727	Swelling reported week 76--tail Not reported week 80
2M	83737	Wart-like lesion reported week 92--back Not reported week 100
2M	83751	Nodule reported week 72--lower midline Not reported week 84 Reported week 92--left inguinal Not reported week 100

<u>Group</u>	<u>Animal Number</u>	<u>Observation</u>
2F	83772	Swelling reported week 64--lower midline Not reported week 76
2F	83773	Swelling reported week 72--mouth Not reported week 76
2F	83774	Swelling reported week 64--right inguinal Not reported week 76 Reported as nodule week 80--right inguinal Not reported week 92 Nodule reported week 76--right axilla Not reported week 80 Reported week 92--right axilla
2F	83780	Nodule reported day 49--chest Not reported day 52 Reported week 80--right axilla
2F	83784	Swelling reported week 72--right inguinal Not reported week 76
2F	83785	Swelling reported week 84--right inguinal Not reported week 88
2F	83786	Nodule reported week 60--right inguinal Not reported week 84 Reported week 88--right inguinal Nodule reported week 88--left axilla Not reported week 100
2F	83787	Nodule reported week 64--right inguinal Not reported week 84 Nodule reported week 64--right axilla Not reported week 92
2F	83792	Nodule reported week 44--left inguinal Not reported week 56 Reported week 68--right inguinal Not reported week 80 Reported week 100--left inguinal
2F	83793	Swelling reported week 76--neck Not reported week 80
2F	83805	Swelling reported week 72--left hind leg Not reported week 84
3M	83810	Swelling reported week 68--mouth Not reported week 72
3M	83822	Swelling reported week 34--left hind leg Not reported week 76
3M	83826	Swelling reported week 38--right ear Not reported week 76 Reported as tissue mass week 104--right side of back
3M	83831	Swelling reported week 48--mouth Not reported week 52
3M	83832	Wart-like lesion reported week 88--left front paw Not reported week 92 Wart-like lesion reported week 92--right front paw Not reported week 100
3M	83839	Swelling reported day 42--neck Not reported day 49
3M	83845	Swelling reported week 64--mouth Not reported week 68
3F	83850	Wart-like lesion reported week 42--right flank, right side of back Not reported week 52

Appendix IV-3
page 3

<u>Group</u>	<u>Animal Number</u>	<u>Observation</u>
3F	83855	Swelling reported day 59--neck Not reported day 63
3F	83861	Swelling reported week 52--lower midline Not reported week 84 Reported as nodule week 96--right inguinal Nodule reported week 60--left axilla Not reported week 72 Reported week 84--right axilla
3F	83863	Nodule reported week 84--right inguinal Not reported week 88
3F	83868	Wart-like lesion reported week 92--right ear Not reported week 100
3F	83873	Swelling reported week 76--lower midline Not reported week 84 Reported as nodule week 100--right inguinal
3F	83883	Nodule reported week 64--left axilla, right and left inguinal Not reported week 68
4M	83889	Nodule reported week 88--lower midline Not reported week 92
4M	83910	Swelling reported week 52--right hind leg Not reported week 64
4M	83918	Wart-like lesion reported week 68--back Not reported week 88
4F	83930	Swelling reported week 50--lower midline Not reported week 64 Reported as nodule week 80--anus
4F	83931	Nodule reported week 84--anus, right and left axilla Not reported week 88 Reported week 104--left axilla, right inguinal
4F	83932	Nodule reported week 50--left inguinal Not reported week 68
4F	83933	Swelling reported week 100--mouth Not reported week 104
4F	83934	Swelling reported week 60--right inguinal Not reported week 76 Reported as nodule week 84--right inguinal
4F	83938	Swelling reported week 72--left inguinal Not reported week 80 Reported as nodule week 92--left inguinal
4F	83939	Swelling reported week 64--right inguinal Not reported week 92 Nodule reported week 72--left axilla Not reported week 80
4F	83941	Nodule reported week 88--left inguinal Not reported week 92 Reported week 100--left axilla
4F	83943	Nodule reported week 44--left inguinal Not reported week 72
4F	83948	Nodule reported week 64--left inguinal Not reported week 84
4F	83958	Nodule reported week 44--chest Not reported week 60 Reported week 76--left axilla
4F	83961	Swelling reported week 64--left inguinal Not reported week 88 Reported as nodules week 104--right inguinal Nodule reported week 84--right axilla Not reported week 88

<u>Group</u>	<u>Animal Number</u>	<u>Observation</u>
4F	83962	Nodule reported week 50--left axilla Not reported week 72 Reported week 76--left axilla Not reported week 80
4F	83964	Nodule reported week 64--left inguinal Not reported week 76
5M	83973	Nodule reported week 52--lower midline Not reported week 68
5M	83976	Nodule reported week 28--lower midline Not reported week 36
5M	83979	Nodule reported week 68--right inguinal Not reported week 72
5M	83988	Nodule reported week 92--lower midline Not reported week 100
5M	83992	Nodule reported week 52--right axilla Not reported week 56
5M	84002	Nodule reported week 80--lower midline Not reported week 98 Reported week 104
5F	84011	Nodule reported week 60--right and left inguinal Not reported week 72
5F	84012	Nodule reported week 40--lower midline Not reported week 42 Reported as tissue mass week 72--chest, left axilla
5F	84014	Swelling reported week 68--mouth Not reported week 76 Reported as nodule week 80--right axilla
5F	84015	Nodule reported week 72--right and left axilla Not reported week 80 Nodule reported week 80--right inguinal Not reported week 88
5F	84023	Swelling reported week 72--left axilla Not reported week 76 Swelling reported week 76--right inguinal Not reported week 88
5F	84024	Nodule reported week 56--lower midline Not reported week 84 Nodule reported week 72--left axilla Not reported week 76
5F	84026	Nodule reported week 72--left axilla Not reported week 76 Reported week 80--left axilla
5F	84033	Nodule reported week 46--right inguinal Not reported week 72
5F	84034	Nodule reported week 80--left inguinal Not reported week 88
5F	84037	Swelling reported week 72--left inguinal Not reported week 92

APPENDIX IV-4

SCHEDULE FOR ADJUSTMENT OF ASPARTAME CONSUMPTION

Protocol Frequency		Actual Frequency	
<u>Interval</u>	<u>Frequency</u>	<u>Interval</u>	<u>Frequency</u>
0-4 week	3 per week	0-5 week	3 per week
5-13 week	2 per week	6-15 week	2 per week
14-26 week	1 per week	16-25 week	1 per week
27-52 week	biweekly	27-52 week	Every 2 weeks
53-termination	monthly	53-104 week	Every 4 weeks

APPENDIX IV-5

STATISTICALLY SIGNIFICANT DIFFERENCES BETWEEN BODY WEIGHTS OF GROUPS AT
VARIOUS INTERVALS BASED ON ANALYSIS OF VARIANCE (ANOVA), LEAST SIGNIFICANT
DIFFERENCE (LSD), AND NEWMAN-KEULS (Q) METHODS AT $P < 0.05$.

Interval Week	Sex	ANOVA	5<4	5<3	5<2	5<1	4<3	4<2	4 vs 1	3 vs 2	3 vs 1	2>1
4	M	0.13	Ø	Ø	Ø	Ø	Ø	Ø	Ø	Ø	Ø	Ø
4	F	0.45	Ø	Ø	Ø	Ø	Ø	Ø	Ø	Ø	Ø	Ø
8	M	0.65	Ø	Ø	Ø	Ø	Ø	Ø	Ø	Ø	Ø	Ø
8	F	0.28	Ø	Ø	Ø	Ø	Ø	Ø	Ø	Ø	Ø	Ø
12	M	0.49	Ø	Ø	Ø	Ø	Ø	Ø	Ø	Ø	Ø	Ø
12	F	0.00	Ø	+	+	+	Ø	+	Ø	Ø	Ø	Ø
20	M	0.42	Ø	Ø	Ø	Ø	Ø	Ø	Ø	Ø	Ø	Ø
20	F	0.01	Ø	+	+	Ø	Ø	Ø	Ø	Ø	Ø	Ø
26	M	0.01	+	+	+	+	Ø	Ø	Ø	Ø	Ø	Ø
26	F	0.00	+	+	+	+	+	Ø	Ø	Ø	Ø	Ø
34	M	0.00	+	+	+	+	Ø	Ø	Ø	Ø	Ø	Ø
34	F	0.00	+	+	+	+	+	Ø	Ø	Ø	Ø	Ø
42	M	0.00	+	+	+	+	Ø	Ø	Ø	Ø	Ø	Ø
42	F	0.00	+	+	+	+	Ø	Ø	Ø	Ø	Ø	Ø
50	M	0.00	+	+	+	+	Ø	Ø	Ø	Ø	Ø	Ø
50	F	0.00	+	+	+	+	Ø	+	Ø	Ø	Ø	±
52	M	0.00	+	+	+	+	Ø	Ø	Ø	Ø	Ø	Ø
52	F	0.00	+	+	+	+	Ø	±	Ø	Ø	Ø	±
60	M	0.00	+	+	+	+	Ø	Ø	Ø	Ø	Ø	Ø
60	F	0.00	+	+	+	+	±	+	Ø	Ø	Ø	±
68	M	0.00	+	+	+	+	Ø	Ø	Ø	Ø	Ø	Ø
68	F	0.00	+	+	+	+	Ø	±	Ø	Ø	Ø	±
84	M	0.00	+	+	+	+	Ø	Ø	Ø	Ø	Ø	Ø
84	F	0.00	+	+	+	+	Ø	±	Ø	Ø	Ø	Ø
92	M	0.00	+	+	+	+	Ø	Ø	Ø	Ø	Ø	Ø
92	F	0.09	Ø	Ø	Ø	Ø	Ø	Ø	Ø	Ø	Ø	Ø
100	M	0.01	Ø	+	+	+	Ø	Ø	Ø	Ø	Ø	Ø
100	F	0.23	Ø	Ø	Ø	Ø	Ø	Ø	Ø	Ø	Ø	Ø
104	M	0.00	+	+	+	+	Ø	Ø	Ø	Ø	Ø	Ø
104	F	0.13	Ø	Ø	Ø	Ø	Ø	Ø	Ø	Ø	Ø	Ø

ANOVA indicates the exact probability that all group means are equal, based upon the F test for Analysis of Variance. UAREP applied the Analysis of Variance at a difference of $p < 0.05$. When values less than 0.05 were obtained the least significant difference (LSD) and Newman-Keuls (Q) tests were run. If the F statistic was greater than 0.05, we did not do the LSD or Q tests because we accept the hypothesis (at the 5% level) that all the means being compared are equal.

Ø means not significant or LSD and Q not done because ANOVA > 0.05.

+

± means LSD significant, but Q not significant.

All ANOVA values of 0.00 in this report indicate less than 1% chance that means are equal.

APPENDIX IV-6A
FEMALE RATS IN E-33, 34 FROM WHOM BLOOD WAS COLLECTED FOR HEMATOLOGY (H)
OR CLINICAL CHEMISTRY (C) SPECIMENS AT INTERVALS OF
13, 26, 52, AND 104 WEEKS

	Interval (weeks)			
	13	26	52	104
Group 1F	83666H -	666H -	666H D	672H
	83667H -	667H -	667H -	667H
	83668H -	668H -	668H -	668H
	83669H -	669H -	669H -	669H
	83670H -	670H -	670H D	674H
Group 1F	83671C -	671C -	671C D	677C
	83672C -	672C -	672C to H	678C
	83673C -	673C -	673C D	679C
	83674C -	674C -	674C to H	681C
	83675C -	675C -	675C D	682C
Group 2F	83766H -	766H -	766H D	771H
	83767H -	767H -	767H D	773H
	83768H -	768H -	768H -	768H
	83769H -	769H -	769H -	769H
	83770H -	770H -	770H -	779H
Group 2F	83771C -	771C -	771C to H	780C
	83772C -	772C -	772C D	781C
	83773C -	773C -	773C to H	783C
	83774C -	774C -	774C D	784C
	83775C -	775C to H	776C D	785C
Group 3F	83846H -	846H -	846H -	846H
	83847H -	847H -	847H -	847H
	83848H -	848H -	848H D	854H
	83849H -	849H -	849H D	855H
	83850H -	850H -	850H -	850H
Group 3F	83851C -	851C -	851C D	868C
	83852C -	852C D	859C D	870C
	83853C -	853C -	853C -	872C
	83854C -	854C to H	856C D	873C
	83855C -	855C -	855C to H	874C
Group 4F	83926H -	926H -	926H D	932H
	83927H -	927H -	927H -	927H
	83928H -	928H -	928H D	933H
	83929H -	929H D	931H -	931H
	93830H -	930H -	930H D	938H
Group 4F	83931C -	931C to H	936C D	941C
	83932C -	932C to H	937C to H	942C
	83933C -	933C to H	938C C	947C
	83934C -	934C -	934C D	949C
	83935C -	935C -	935C D	950C
Group 5F	84006H -	006H -	006H D	021H
	84007H -	007H -	007H D	016H
	84008H -	008H -	008H D	017H
	84009H -	009H -	009H -	009H
	84010H -	010H -	010H D	024H
Group 5F	84011C D	016C -	016C to H	036C
	84012C -	012C -	012C D	037C
	84013C -	013C D	017C to H	041C
	84014C -	014C -	014C D	043C
	84015C -	015C -	015C D	045C

After the first 13 week interval, only the last three digits of mouse numbers are indicated. An - indicates same rat used at next interval; rat numbers followed by D, denoting death, or "to H", indicating transfer to hematology tests, were replaced by another rat at the next testing interval.

APPENDIX IV-68

MALE RATS IN E-33, 34 FROM WHICH BLOOD WAS COLLECTED FOR HEMATOLOGY (H)
OR CLINICAL CHEMISTRY (C) SPECIMENS AT INTERVALS OF 13, 26, 52, AND 104 WEEKS

	<u>Interval (weeks)</u>			
	<u>13</u>	<u>26</u>	<u>52</u>	<u>104</u>
Group 1M	83606H → 606H → 606H D 618H			
	83607H → 607H → 607H → 607H			
	83608H → 608H D 612H D 620H			
	83609H → 609H D 613H → 613H			
	83610H → 610H D 614H D 621H			
Group 1M	83611C → 611C D 619C D 622C			
	83612C → 612C to H 616C D 624C			
	83613C → 613C to H 617C D 625C			
	83614C → 614C to H 618C to H 626C			
	83615C → 615C → 615C D 627C			
Group 2M	83726H → 726H D 731H → 731H			
	83727H → 727H → 727H → 727H			
	83728H → 728H → 728H D 732H			
	83729H → 729H → 729H D 737H			
	83730H → 730H → 730H D 739H			
Group 2M	83731C → 731C to H 736C D 743C			
	83732C → 732C to H 737C to H 744C			
	83733C → 733C → 733C D 745C			
	83734C → 734C → 734C D 746C			
	83735C → 735C → 735C D 750C			
Group 3M	83806H → 806H → 806H → 806H			
	83807H → 807H → 807H → 807H			
	83808H → 808H D 811H D 813H			
	83809H → 809H → 809H → 814H			
	83810H → 810H → 810H → 810H			
Group 3M	83811C → 811C to H 816C D 822C			
	83812C → 812C → 812C D 823C			
	83813C → 813C → 813C to H 825C			
	83814C → 814C → 814C to H 826C			
	83815C → 815C → 815C D 827C			
Group 4M	83886H → 886H D 891H D 893H			
	83887H → 887H → 887H → 887H			
	83888H → 888H → 888H D 910H			
	83889H → 889H → 889H → 889H			
	83890H → 890H → 890H → 890H			
Group 4M	83891C → 891C to H 896C D 903C			
	83892C → 892C D 899C → 899C			
	83893C → 893C → 893C to H 901C			
	83894C → 894C D 898C → 898C			
	83895C → 895C → 895C D 904C			
Group 5M	83966H → 966H → 966H → 966H			
	83967H → 967H → 967H D 972H			
	83968H → 968H → 968H D 977H			
	83969H → 969H → 969H D 978H			
	83970H → 970H → 970H → 970H			
Group 5M	83971C → 971C → 971C D 980C			
	83972C → 972C → 972C to H 981C			
	83973C → 973C → 973C D 983C			
	83974C → 974C → 974C D 984C			
	83975C → 975C → 975C D 988C			

After the first 13 week interval, only the last three digits of mouse numbers are indicated. An → indicates same rat used at next interval; rat numbers followed by D, denoting death, or "to H", indicating transfer to hematology tests, were replaced by another rat at the next testing interval.

APPENDIX IV-7

SUMMARY OF DISCREPANCIES IN HEMATOLOGY NOTED IN

APPENDIX TABLE NO. 2 OF ENTRY BOOK E-33, pp 16-31

Interval (week)	Parameter	Group	HLA Value	Type of Discrepancy	UAREP Value
13	Hct	2M	± 5.23	R	5.22 (5.224)
13	WBC	3M	S ⁻	ST	See Appendix IV-8
26	RBC	1M	± 0.81	R	0.80 (0.8053)
52	Hct	2M	± 1.25	R	1.24 (1.245)
52	Hgb	3M	± 0.20	R	0.19 (0.1949)
52	RBC	5M	± 0.59	R	0.58 (0.5853)
104	Hgb	2M	± 2.07	R	2.06 (2.0652)
104	WBC	2M	± 8.83	R	8.84 (8.835)
13	RBC	3F	± 0.51	R	0.50 (0.5054)
13	WBC	5F	± 2.46	R	2.45 (2.4550)
104	Hgb	2F	± 1.21	R	1.20 (1.2054)
104	RBC	5F	S ⁺	ST	See Appendix IV-8
104	WBC	5F	S ⁻	ST	See Appendix IV-8

All of the above inconsequential rounding (R) discrepancies involved standard deviations and would not alter interpretation of results. UAREP and HLA agreed on the results of all t-tests run by both, but UAREP applied other tests shown in Appendix IV-8.

APPENDIX IV-3
COMPARISON OF UAREP AND HLA STATISTICALLY SIGNIFICANT HEMATOLOGY
GROUP DIFFERENCES

Parameter	Interval	Sex	ANOVA	Groups	Q	LSD	UAREP t-test	t-test value	HLA t-test
WBC	13	M	.22	1>3	ND	ND	S	2.38	S ⁻
		M		1>3	S	S	S	3.00	S ⁻
	26	M	.01	1>4	S	S	S	2.34	S ⁻
				1>5	N	S	S	2.70	S ⁻
				2>3	S	S	S	2.77	ND
				2>4	S	S	N	--	ND
				2>5	N	S	S	2.46	ND
	52	M	.10	2>5	ND	ND	S	5.24	ND
	104	F	.51	1>5	ND	ND	S	2.54	S ⁻
Hct	52	M	.02	1>3	N	S	N	--	N
				3<4	S	S	S	2.99	ND
				4>5	N	S	S	4.36	ND
Hgb	26	M	.07	2<5	ND	ND	S	2.85	ND
RBC	26	F	.00	1>2	S	S	S	4.16	S ⁻
				1>3	S	S	S	2.33	S ⁻
				1>5	S	S	S	2.72	S ⁻
				2<4	S	S	S	4.93	ND
				3<4	N	S	N	--	ND
	52	F	.22	2<3	ND	ND	S	2.79	ND
	104	F	.07	1<5	ND	ND	S	2.43	S ⁺

ANOVA indicates the exact probability that all group means are equal, based upon the F test for Analysis of Variance. UAREP applied the Analysis of Variance at a difference of $p < 0.05$. When values less than 0.05 were obtained the least significant difference (LSD) and Newman-Keuls (Q) tests were run. If the F statistic was greater than 0.05, we did not do the LSD or Q tests because we accept the hypothesis (at the 5% level) that all the means being compared are equal.

S means $p < 0.05$.

N means $p > 0.05$.

UAREP used the two tailed t-test with 8 degrees of freedom, $t_{0.05} = 2.306$. HLA has not specified their critical value for $t_{0.05}$. They compared experimental groups with controls whereas UAREP did intergroup analysis comparison of all groups.

ND = not done

APPENDIX IV-9
UAREP COMPUTED CONFIDENCE INTERVALS (P<0.05) BASED ON CONTROL
HEMATOLOGY GROUP VALUES FOR E-33,34

<u>Hematocrit</u>						
Male Groups						
Interval (weeks)	1	2	3	4	5	Confidence Interval
13	47.4	47.4	48.5	48.2	46.8	44.98 - 49.82
26	46.9	47.5	44.7	46.2	46.8	44.57 - 49.23
52	47.0	46.1	44.5	47.8	45.2	44.52 - 49.48
104	48.0	47.4	48.3	48.4	47.4	37.97 - 58.03
Female Groups						
13	46.6	46.0	47.0	47.7	45.6	45.49 - 47.71
26	46.6	44.0	45.4	46.0	45.8	43.11 - 50.09
52	44.4	43.2	45.8	44.4	42.7	39.88 - 48.92
104	44.8	50.2	45.0	42.8	46.4	34.45 - 55.15

<u>Hemoglobin</u>						
Male Groups						
Interval (weeks)	1	2	3	4	5	Confidence Interval
13	15.66	16.18	17.08	17.00	16.26	14.06 - 17.26
26	15.00	14.28	15.04	15.42	15.76	13.83 - 16.17
52	14.28	13.88	14.06	14.54	14.36	13.39 - 15.17
104	15.34	15.30	15.86	15.78	15.82	12.07 - 18.61
Female Groups						
13	14.72	14.98	15.20	15.74	14.96	13.37 - 16.07
26	15.18	14.08	14.92	15.12	14.56	13.69 - 16.67
52	13.74	13.00	14.28	14.40	14.16	12.25 - 15.23
104	13.92	16.34	15.24	13.64	15.22	10.01 - 17.83

<u>RBC</u>						
Male Groups						
Interval (weeks)	1	2	3	4	5	Confidence Interval
13	7.75	7.86	8.13	7.94	7.80	7.08 - 8.42
26	6.44	6.01	6.13	6.09	6.38	5.44 - 7.44
52	7.55	7.44	7.08	7.58	7.20	7.29 - 7.81
104	7.60	7.68	8.16	8.01	8.17	5.94 - 9.26
Female Groups						
13	7.97	7.62	7.63	7.84	7.54	7.54 - 8.40
26	6.69	5.09	5.55	6.32	5.78	5.80 - 7.58
52	6.72	6.30	7.14	7.01	6.57	5.93 - 7.51
104	6.53	7.68	7.04	6.15	8.28	4.90 - 8.16

<u>WBC</u>						
Male Groups						
Interval (weeks)	1	2	3	4	5	Confidence Interval
13	21.08	21.94	16.42	21.00	16.44	17.07 - 25.09
26	24.58	23.46	13.88	15.94	15.20	16.25 - 32.91
52	18.08	20.92	15.02	18.68	12.40	10.84 - 25.32
104	18.88	22.32	17.96	19.28	15.36	15.64 - 22.12
Female Groups						
13	13.86	14.46	13.76	14.62	12.42	9.96 - 17.76
26	18.02	16.36	15.24	15.50	13.02	12.13 - 23.91
52	17.18	15.70	11.56	12.92	19.52	11.05 - 23.31
104	21.12	21.08	18.38	18.10	14.62	15.07 - 27.17

APPENDIX IV-10

DISCREPANCIES IN BLOOD CHEMISTRIES IN APPENDIX TABLE NO. 3

OF ENTRY BOOK E-33, pp 32-47

<u>Interval (week)</u>	<u>Parameter</u>	<u>Group</u>	<u>HLA Value</u>	<u>Type Of Discrepancy</u>	<u>UAREP Value</u>
13	Alk. Phos.	1M	±5.357	R	5.356 (5.3565)
13	BUN	3M	S ⁻	ST	See Appendix IV-11
13	Glucose	4M	±5.59	R	5.58 (5.5857)
13	SGPT	5M	±35.37	R	35.36 (35.3562)
26	SGPT	1M	±6.03	R	6.02 (6.0249)
52	SGPT	3M	±5.81	R	5.80 (5.8052)
52	Alk. Phos.	1M	±6.531	R	6.530 (6.5305)
52	SGPT	4M	±6.69	R	6.68 (6.6858)
104	SGOT	1M	±16.119	R	16.118 (16.1183)
104	Total Bilirubin	5M	S ⁺	ST	See Appendix IV-11
104	Total Bilirubin	5M	±0.005	R	0.004 (0.0045)
13	SGPT	1F	±5.81	R	5.80 (5.8052)
13	Total Bilirubin	2F	S ⁺	ST	See Appendix IV-11
13	Alk. Phos.	5F	±5.493	R	5.492 (5.4925)
26	Glucose	1F	±9.99	R	9.98 (9.985)
52	Glucose	5F	S ⁻	ST	See Appendix IV-11
104	SGOT	3M	S ⁺	ST	See Appendix IV-11
104	Total Bilirubin	1F	±0.005	R	0.004 (0.0045)
104	SGPT	3F	S ⁻	ST	See Appendix IV-11
104	SGPT	5F	S ⁺	ST	See Appendix IV-11

Appendix IV-10
(cont'd) page two

All of the inconsequential rounding (R) discrepancies involved standard deviations and would not alter interpretation of results. The statistical (S) discrepancies did not involve the t-test applied by both HLA and UAREP, but other tests of significance applied only by UAREP.

APPENDIX IV-11 (cont.)
page two

Parameter	Inter- val	Sex	ANOVA	Groups	Q	LSD	UAREP t-test	t-test value	HLA t-test
CO ₂	104	M	.01	1>3	S	S	N	--	N
				2>3	S	S	N	--	ND
				3<4	S	S	N	--	ND
				3<5	S	S	S	2.80	ND
		F	.07	1<4	ND	ND	N	--	N
				4>5	ND	ND	S	2.58	ND
L-Phenyl- alanine	104	M	.01	1>4	S	S	S	2.54	S
				1>5	S	S	S	3.52	S

ANOVA indicates the exact probability that all group means are equal, based upon the F test for Analysis of Variance. UAREP applied the Analysis of Variance at a difference of $p < 0.05$. When values less than 0.05 were obtained the least significant difference (LSD) and Newman-Keuls (Q) tests were run. If the F statistic was greater than 0.05, we did not do the LSD or Q tests because we accept the hypothesis (at the 5% level) that all the means being compared are equal.

S means $p < 0.05$.

N means $p > 0.05$

UAREP used the two tailed t-test with 8 degrees of freedom, $t_{0.05} = 2.306$. HLA has not specified their critical value for $t_{0.05}$. They compared experimental groups with controls whereas UAREP did intergroup analysis comparison of all groups.

ND = not done

APPENDIX IV-12

WAREP COMPUTED CONFIDENCE INTERVALS (P<0.05) FOR CLINICAL CHEMISTRIES FOR E-33,34

Glucose (mg/dl)						
Interval (week)	Male Groups					Confidence Interval
	1	2	3	4	5	
13	102.0	117.0*	109.4	109.2	107.0	92.9 - 111.1
26	77.8	101.8*	82.6	83.6	86.8	66.7 - 88.9
52	115.0	114.6	109.0	110.6	89.4*	99.9 - 130.1
104	123.0	123.0	137.2	117.6	126.0	96.7 - 149.3
Female Groups						
13	109.4	107.8	104.0	99.8	108.2	97.1 - 121.7
26	109.2	109.8	94.0*	88.6*	100.8	96.8 - 121.6
52	117.6	108.8	115.8	105.8	95.2*	101.7 - 133.5
104	121.2	133.2	124.6	123.4	122.6	94.7 - 147.7
BUN (mgm %)						
Male Groups						
13	16.4	14.6	13.6*	15.0	15.6	14.2 - 18.6
26	15.2	16.6	13.4	15.4	13.0	11.8 - 18.6
52	16.2	16.0	16.2	17.0	17.2	14.8 - 17.6
104	15.5	16.0	19.0*	15.5	18.9*	13.8 - 17.2
Female Groups						
13	16.6	18.6*	17.4	16.6	17.2	14.9 - 18.3
26	18.6	20.2	18.6	18.2	18.4	14.5 - 22.7
52	18.2	20.2*	18.2	17.4	18.8	16.4 - 20.0
104	16.0	16.1	15.2	15.1	19.4	10.1 - 21.9
SGPT (R-F units)						
Male Groups						
13	46.3	57.6*	42.0	40.8	65.2	40.1 - 52.5
26	40.4	49.6*	38.2	39.2	35.6	32.9 - 47.9
52	33.4	39.2	32.8	35.8	35.4	25.2 - 41.6
104	30.2	35.8	34.0	32.8	33.2	24.6 - 35.0
Female Groups						
13	52.8	54.8	49.7	48.8	45.3*	45.6 - 60.0
26	46.0	43.0	40.8	42.6	40.2	34.5 - 57.5
52	36.6	39.0	30.4	28.4*	36.0	29.6 - 43.6
104	33.6	36.2	29.4	35.2	40.8*	29.4 - 37.0

Appendix IV-12 (cont'd)
page two

Alkaline Phosphatase (K-A units)

Interval (week)	<u>Male Groups</u>					Confidence Interval
	1	2	3	4	5	
13	21.5	28.3*	26.0	24.0	27.5	14.8 - 28.1
26	16.6	30.4*	21.0	21.1	16.6	11.9 - 21.2
52	17.0	22.7	18.0	20.8	18.9	8.9 - 25.0
104	22.1	24.1	25.8	20.7	17.1	9.6 - 34.6

<u>Female Groups</u>						
13	18.2	18.3	24.8	20.0	20.3	9.4 - 27.1
26	15.0	15.7	17.8*	13.1	14.4	12.9 - 17.2
52	18.3	22.3	21.2	14.6	14.6	11.7 - 24.9
104	18.4	15.8	13.7	18.7	17.5	10.0 - 26.8

Total Bilirubin (mgm %)

<u>Male Groups</u>						
13	0.81	0.80	1.01	0.97	1.03	0.52 - 1.10
26	0.55	0.60	0.54	0.55	0.57	0.44 - 0.66
52	0.14	0.15	0.15	0.15	0.14	0.11 - 0.17
104	0.34	0.47*	0.33	0.36	0.40	0.28 - 0.40

<u>Female Groups</u>						
13	0.91	1.16*	1.13*	1.01	1.00	0.79 - 1.03
26	0.59	0.57	0.55	0.57	0.57	0.50 - 0.68
52	0.15	0.14	0.14	0.16	0.16	0.12 - 0.18
104	0.40	0.40	0.44*	0.44*	0.38*	0.39 - 0.41

* Mean values falling outside confidence interval

APPENDIX IV-13

UAREP NOTED DISCREPANCIES IN ELECTROPHORESIS DATA CONTAINED IN

APPENDIX TABLE 3 ENTRY BOOK E-33, pp 48-63

<u>Interval (weeks)</u>	<u>Parameter</u>	<u>Group</u>	<u>HLA Value</u>	<u>Type of Discrepancy</u>	<u>UAREP Value</u>
26	Total Protein	5M	± 1.149	R	.148 (.1483)
26	Alpha 1	1M	18.2	T	8.2 (earliest data source)
26	Alpha 1	1M	18.54	C	16.54
26	Alpha 1	1M	± 2.59	C	5.33
26	Albumin	4M	± 2.95	R	2.94 (2.9453)
52	Gamma	5M	S ⁻	ST	See Appendix IV-14
104	Alpha 1	3M	S ⁺	ST	See Appendix IV-14
104	Alpha 1	5M	± 2.35	R	2.34 (2.3452)
104	Gamma	5M	S ⁻	ST	See Appendix IV-14
13	Beta	2F	S ⁻	ST	See Appendix IV-14
13	Alpha 1	3F	± 1.47	R	1.46 (1.4656)
13	Alpha 2	4F	± 3.89	R	3.88 (3.8859)
26	Alpha 1	1F	± 1.75	R	1.74 (1.7456)
26	Gamma	1F	± 2.17	R	2.16 (2.1652)
26	Beta	4F	S ⁺	ST	See Appendix IV-14
52	Albumin	1F	± 1.03	R	1.02 (1.0247)
52	Albumin	3F	S ⁺	ST	None
104	Beta	3F	S ⁻	ST	See Appendix IV-14
104	Alpha 1	4F	S ⁺	ST	See Appendix IV-14
104	Gamma	4F	S ⁺	ST	See Appendix IV-14
104	Total Protein	4F	± 0.327	R	0.326 (0.3264)

Appendix IV-13
(cont'd) page two

All of the inconsequential rounding (R) discrepancies involved standard deviations and would not alter interpretation of the results. In five of the eight statistical (S) discrepancies HLA & UAREP agreed on t-test significance.

APPENDIX IV-14
COMPARISON OF UAREP AND HLA STATISTICALLY SIGNIFICANT GROUP DIFFERENCES
IN BLOOD PROTEIN DETERMINATIONS

Parameter	Inter- val	Sex	ANOVA	Groups	UAREP	UAREP	t-test	HLA
					UAREP	t-test	value	t-test
Total Protein	13	F	.00	1<2	S	S	4.95	S ⁺
				1<4	S	S	--	S ⁻
				1>5	S	S	4.67	S ⁻
				2>3	S	S	2.72	ND
				2>4	S	S	4.63	ND
				2>5	S	S	9.33	ND
				3>4	S	S	2.57	ND
				3>5	S	S	3.93	ND
	26	M	.04	2>3	S	S	2.87	ND
				2>4	N	S	--	ND
				2>5	S	S	3.29	ND
	52	M	.19	2>5	ND	ND	2.40	ND
Albumin	26	M	.01	2<4	N	S	2.84	ND
				2<5	S	S	4.83	ND
				3<5	N	S	3.51	ND
	52	M	.00	1<2	S	S	(2.25)	S ⁻
				1<5	S	S	3.88	S ⁺
				2<3	S	S	2.92	ND
				2<4	S	S	3.83	ND
				2<5	S	S	5.31	ND
				3<5	N	S	2.65	ND
	52	F	.01	1>2	N	S	--	N ⁺
				1<3	N	N	3.25	S ⁺
				2<3	S	S	3.67	ND
				2<5	N	S	--	ND
				3>4	S	S	3.33	ND
	104	M	.01	1>3	S	S	2.33	S ⁻
				2>3	N	S	2.62	ND
				3<5	S	S	4.51	ND
				4<5	N	S	3.02	ND
Alpha 1	104	M	.16	1<3	ND	ND	2.48	S ⁺
	104	F	.14	1<4	ND	ND	2.55	S ⁺
				4>5	ND	ND	2.73	ND
Alpha 2	52	F	.09	2>3	ND	ND	(2.29)	ND
				2>4	ND	ND	--	ND
	104	M	.07	3>4	ND	ND	2.33	ND
				3>5	ND	ND	3.00	ND
Beta	13	F	.19	1>2	ND	ND	(2.20)	S ⁻
	26	F	.17	1<4	ND	ND	--	S ⁺
	52	M	.01	1<2	S	S	--	N
				2>3	S	S	--	ND
				2>4	S	S	2.61	ND
				2>5	S	S	2.67	ND
	104	M	.05	1<4	N	S	--	N
				2<4	N	S	--	ND
				4>5	S	S	--	ND
		F	.08	1>3	ND	ND	2.47	S ⁻
				1>4	ND	ND	--	N

APPENDIX IV-14 (cont.)
page two

Parameter	Inter- val	Sex	ANOVA	Groups	Q	LSD	UAREP t-test	t-test value	HLA t-test
Gamma	26	M	.04	2<3	N	S	N	--	ND
				3>4	S	S	S	2.69	ND
				3>5	S	S	S	2.47	ND
	52	M	.03	1>5	N	N	S	2.59	S ⁻
				2>3	N	S	S	2.61	ND
				2>4	N	S	N	--	ND
				2>5	S	S	S	4.25	ND
	104	M	.09	1>5	ND	ND	S	2.42	S ⁻
				2>5	ND	ND	S	3.28	ND
		F	.13	1<4	ND	ND	S	4.15	S ⁺

ANOVA indicates the exact probability that all group means are equal, based upon the F test for Analysis of Variance. UAREP applied the Analysis of Variance at a difference of $p < 0.05$. When values less than 0.05 were obtained the least significant difference (LSD) and Newman-Keuls (Q) tests were run. If the F statistic was greater than 0.05, we did not do the LSD or Q tests because we accept the hypothesis (at the 5% level) that all the means being compared are equal.

S means $p < 0.05$.

N means $p > 0.05$.

UAREP used the two tailed t-test with 8 degrees of freedom, $t_{0.05} = 2.306$. HLA has not specified their critical value for $t_{0.05}$. They compared experimental groups with controls whereas UAREP did intergroup analysis comparison of all groups.

ND = not done

APPENDIX IV-15

UAREP COMPUTED CONFIDENCE INTERVALS (P<0.05) FOR
BLOOD ELECTROPHORESIS FOR E-33,34

Total Serum Protein, Expressed as Grams %

Male Groups

Interval (weeks)	1	2	3	4	5	Confidence Intervals
13	7.30	6.87	6.90	6.52	6.70	6.27 - 8.33
26	7.48	8.22	7.16	7.42	7.08	6.55 - 8.41
52	6.26	6.98	5.82	6.36	5.58	4.77 - 7.75
104	6.16	6.56	5.81	6.52	6.17	5.43 - 6.89

Female Groups

13	7.30	8.00	7.32	6.70	6.60	6.03 - 8.37
26	8.14	8.24	8.48	7.70	7.68	6.54 - 9.74
52	7.00	7.64	6.90	6.94	6.48	6.20 - 7.80
104	6.82	6.96	6.10	6.34	6.58	6.06 - 7.58

Albumin, Expressed as Percent of Total Serum Protein

Male Groups

Interval (weeks)	1	2	3	4	5	Confidence Intervals
13	46.1	43.5	47.2	46.8	42.9	38.2 - 54.0
26	44.8	40.9	43.4	45.9	49.2	38.3 - 51.3
52	38.4	31.8*	40.5	42.5	45.6*	33.9 - 42.9
104	37.4	35.2	26.8*	31.0	41.4	27.2 - 47.6

Female Groups

13	47.8	46.1	50.1	50.5	50.8	44.7 - 50.9
26	51.0	49.1	52.5	51.8	50.9	47.3 - 54.7
52	45.9	42.3	49.0*	43.7	46.6	44.6 - 47.2
104	41.4	36.6	43.6	36.6	43.4	30.6 - 52.2

Alpha 1, Expressed as Percent of Total Serum Protein

Male Groups

Interval (weeks)	1	2	3	4	5	Confidence Intervals
13	18.3	18.2	17.8	17.8	17.5	14.9 - 21.7
26	16.5	18.3	18.7	20.6	18.0	9.9 - 23.2
52	21.6	21.0	21.5	21.0	20.1	20.2 - 23.0
104	13.6	15.5	20.4*	16.4	16.0	9.5 - 17.7

Female Groups

13	16.8	16.1	18.2	16.1	17.8	14.9 - 18.7
26	15.9	16.5	15.1	14.5	15.6	13.8 - 18.1
52	17.3	18.7	17.8	20.5*	16.6	15.3 - 19.3
104	10.4	12.4	13.7*	15.0*	10.9	7.3 - 13.5

Alpha 2, Expressed as Percent of Total Serum Protein

Male Groups

Interval (weeks)	1	2	3	4	5	Confidence Intervals
13	13.5	15.5	12.1	13.2	17.1	5.4 - 21.6
26	11.8	14.3	11.7	12.1	10.2	6.6 - 17.0
52	13.0	13.3	12.6	12.0	11.3	8.0 - 18.0
104	11.5	10.8	13.0	10.2	10.0	9.0 - 14.0

Female Groups

13	12.6	16.5	12.3	12.0	10.2	9.2 - 16.0
26	12.6	12.3	9.3	9.8	10.5	7.9 - 17.3
52	11.3	14.2	9.6	10.1	12.3	7.7 - 14.9
104	8.2	9.8	9.1	8.8	9.2	6.2 - 10.2

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continued, page two

Beta⁺, Expressed as Percent of Total Serum Protein

Male Groups						
Interval (weeks)	1	2	3	4	5	Confidence Intervals
13	20.2	20.5	20.5	20.1	19.8	18.2 - 22.1
26	21.2	23.0	21.0	18.5	18.9	16.6 - 25.8
52	20.6	25.5*	20.2	18.8	18.8	18.4 - 22.8
104	28.2	29.4	31.4	36.4*	27.2	24.3 - 32.1
Female Groups						
13	18.9	17.0*	16.8*	17.5	18.6	17.2 - 20.5
26	15.6	17.7	18.7*	18.4	16.6	12.7 - 18.5
52	17.3	17.5	16.3	17.8	17.6	15.7 - 18.9
104	32.6	30.2	22.4*	24.6	26.2	23.3 - 41.9

Gamma, Expressed as Percent of Total Serum Protein

Male Groups						
Interval (weeks)	1	2	3	4	5	Confidence Intervals
13	3.5	4.0	3.5	3.7	2.8	2.2 - 4.8
26	4.9	4.6	6.6*	3.9	4.1	3.6 - 6.2
52	6.3	8.4	5.2	5.7	4.2	4.1 - 8.5
104	9.3	9.1	8.4	6.0	5.4	4.9 - 13.7
Female Groups						
13	5.3	5.1	4.1	4.5	4.5	3.9 - 6.7
26	6.2	4.8	5.8	6.1	6.2	3.5 - 8.8
52	8.2	7.3	7.3	7.9	6.9	4.2 - 12.2
104	7.4	11.0*	11.2*	15.0*	10.3*	4.6 - 10.2

* Denotes Mean Values Outside Confidence Interval

APPENDIX IV-16

DISCREPANCIES IN SERUM LEVELS OF SODIUM, POTASSIUM, CHLORIDE AND
CARBON DIOXIDE AT 104 WEEKS AS CONTAINED IN APPENDIX

TABLE NO. 3 ENTRY BOOK E-33, pp 64-67

<u>Parameter</u>	<u>Group</u>	<u>HLA Value</u>	<u>Type of Discrepancy</u>	<u>Correct Value</u>
Serum K	3M	S ⁺	ST	See Appendix IV-11
CO ₂	3M	±5.75	R	5.74 (5.745)
Serum Na	4M	S ⁺	ST	See Appendix IV-11
Serum Ca	4M	±0.67	R	0.66 (0.6656)
Serum Ca	5M	S ⁺	ST	See Appendix IV-11
CO ₂	5M	±2.09	R	2.08 (2.085)
Serum Na	1F	±1.31	C	1.30 (1.3038)
CO ₂	1F	±2.77	R	2.76 (2.7653)
Serum Na	3F	±1.31	C	1.30 (1.3038)
Serum Na	4F	±0.57	R	0.58 (0.5774)

All of the above inconsequential rounding (R) and computational (C) discrepancies involved standard deviations and would not alter interpretation of results. HLA and UAREP agreed on the t-test significance as applied by both, but UAREP also applied tests of significance not employed by HLA.

APPENDIX IV-17

UAREP COMPUTED CONFIDENCE INTERVALS ($P < 0.05$) BASED ON
CONTROL GROUP VALUES FOR SERUM SODIUM, POTASSIUM, CHLORIDE, CALCIUM,
AND CARBON DIOXIDE AT 104 WEEK INTERVAL OF E-33, PAGES 64-67

<u>Group</u>	<u>Sodium meq/l</u>	<u>Potassium meq/l</u>	<u>Chloride meq/l</u>	<u>Calcium mgm%</u>	<u>Carbon Dioxide meq/l</u>
M1	145.0	3.5	106.5	9.3	27.5
F1	143.8	3.7	104.8	10.0	29.1
M2	144.0*	3.6	106.0	9.8	28.1
F2	144.4	3.3*	102.1*	10.3	30.9
M3	144.6	4.3*	112.0*	10.0*	22.4*
F3	144.8	3.5	105.0	10.1	31.4
M4	146.8*	3.8*	107.5	10.1*	27.8
F4	144.5	3.3*	104.8	10.0	33.6*
M5	148.8*	3.7	106.0	10.0*	30.1*
F5	144.8	3.8	105.1	10.2	28.3
Confidence Interval					
Males	144.1- 145.9	3.3- 3.7	102.5- 110.5	8.9- 9.8	25.2- 29.8
Females	142.2- 145.4	3.5- 3.9	102.5- 107.1	9.7- 10.3	25.7- 32.6

* Denotes mean value outside confidence interval

APPENDIX IV-18

UAREP COMPUTED CONFIDENCE INTERVALS ($P < .05$) BASED ON CONTROL GROUP

VALUES FOR L-PHENYLALANINE (mgm%) AT 104 WEEKS

	Groups					Confidence Interval
	1	2	3	4	5	
Males	2.62	ND	ND	1.68	1.52	1.79-3.45
Females	1.86	ND	ND	1.70	ND	1.40-2.32

ND = No data from determinations obtained by Hazleton.

APPENDIX IV-19

DISCREPANCIES NOTED ON BODY WEIGHTS AND ORGAN TO BODY WEIGHT (BW)
RATIOS IN APPENDIX TABLE NO. 7 (E-33, PAGES 94-99)

Group	Sex	Parameter	HLA Value	Type of Discrepancy	UAREP Value
2	M	Body weight (SD)	±79	R	78 (78.56)
4	M	Body weight	476	C	472
		Body weight (SD)	±88	C	84
5	M	Body weight (N)	20	N*	21
3	F	Body weight (SD)	±59	N	58
5	F	Body weight	302	C	299
		Body weight (SD)	±52	C	54
4	M	Thyroid ratio	0.0074	R	0.0075 (0.00748)
4	F	Thyroid weight		ST	See Appendix 19A
5	F	Thyroid weight (N)	10	N	9
		Thyroid weight		ST	See Appendix 19A
		Thyroid ratio (N)	10	N	9
		Thyroid ratio %	0.0087	N	0.0089
1	M	Heart weight	1.91	R	1.90 (1.905)
4	M	Heart ratio %	0.362	C	0.364
		Heart ratio (SD)	±0.072	C	0.069
5	F	Heart weight	1.18	R	1.20 (1.195)
1	F	Heart ratio %	0.443	R	0.442 (0.4425)
5	F	Heart ratio %	0.399	C	0.406
2	M	Liver weight		ST	See Appendix 19A
3	M	Liver ratio %	3.27	R	3.26 (3.2658)
		Liver ratio %		ST	See Appendix 19A
4	M	Liver ratio %	2.98	C	3.00
		Liver ratio (SD)	±0.47	C	0.46

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(cont'd) page 2

Group	Sex	Parameter	Value	Discrepancy	UAREP Value
5	F	Liver weight	9.87	C	9.03
		Liver weight (SD)	±3.07	C	1.39
		Liver ratio %	3.28	C	3.05
		Liver ratio (SD)	±0.85	C	0.36
1	M	Kidney ratio %	0.800	C	0.798
		Kidney ratio (SD)	±0.167	C	0.168
4	M	Kidney ratio %	0.933	C	0.939
		Kidney ratio (SD)	±0.256	C	0.251
5	F	Kidney weight	2.60	C	2.50
		Kidney weight (SD)	±0.46	C	0.32
		Kidney ratio %	0.879	C	0.855
		Kidney ratio (SD)	±0.179	C	0.170
4	M	Adrenal weight (SD)	±0.017	R	0.016 (0.0165)
1	M	Adrenal ratio %	0.0408	C	0.0407
		Adrenal ratio (SD)	±0.0886	C	0.0884
3	M	Adrenal ratio (SD)	±0.009	R	0.010 (0.0095)
1	F	Adrenal weight (SD)	±0.153	R	0.154 (0.1535)
5	F	Adrenal weight	0.081	C	0.077
		Adrenal weight (SD)	±0.021	C	0.018
		Adrenal weight		ST	See Appendix 19A
		Adrenal ratio %	0.028	C	0.027
2	M	Testis weight	4.81	R	4.80 (4.805)
4	M	Testis weight	4.21	R	4.20 (4.2057)
1	M	Testis ratio %	0.895	C	0.896
		Testis ratio (SD)	±0.227	C	0.228
4	M	Testis ratio %	0.893	C	0.9000
		Testis ratio (SD)	±0.211	C	0.212
1	F	Ovary weight	0.175	C	0.211
		Ovary weight (SD)	±0.186	C	0.236
		Ovary ratio %	0.0559	C	0.0702
		Ovary ratio (SD)	±0.0667	C	0.0912

Appendix IV-19
(cont'd) page 3

Parameter	Sex	ANOVA	Groups	Q	LSD	UAREP t-Test	t-Test Value	HLA Analysis
Seminal vesicles	M	.24	1>4	ND	ND	S	2.02	N
Uterus	F	.02	1<3	N	S	S	2.59	N
			1<4	N	N	S	2.23	N
Uterus/BW	F	.01	1<3	N	S	S	2.48	S ⁺
			1<4	N	N	S	2.80	S ⁺
			2<3	N	S	S	2.54	ND

ANOVA indicates the exact probability that all group means are equal, based upon the F test for Analysis of Variance. UAREP applied the Analysis of Variance at a difference of $P < 0.05$. When values less than 0.05 were obtained the least significant difference (LSD) and Newman-Keuls (Q) tests were run. If the F statistic was greater than 0.05, we did not do the LSD or Q tests because we accept the hypothesis (at the 5% level) that all the means being compared are equal.

S means $P < 0.05$

N means $P > 0.05$

UAREP used the two tailed t-test with 8 degrees of freedom, $t_{0.05} = 2.306$. HLA has not specified their critical value for $t_{0.05}$. They compared experimental groups with controls whereas UAREP did intergroup analysis comparison of all groups.

ND = not done

APPENDIX IV-19A

COMPARISON OF UAREP AND HLA STATISTICALLY SIGNIFICANT GROUP

DIFFERENCES IN ORGAN WEIGHTS AND ORGAN TO

BODY WEIGHT (BW) RATIOS

Parameter	Sex	ANOVA	Groups	Q	LSD	UAREP t-Test	t-Test Value	HLA Analysis
Terminal body weight	M	.00	1>5	S	S	S	4.11	S ⁻
			2>3	N	S	N	-	ND
			2>4	N	S	S	2.32	ND
			2>5	S	S	S	5.04	ND
			3>5	S	S	S	2.47	ND
			4>5	S	S	S	2.50	ND
Thyroid	M	.00	1>5	S	S	S	3.04	S ⁻
			2>3	S	S	S	2.11	ND
			2>4	S	S	S	3.22	ND
			2>5	S	S	S	4.48	ND
			3>5	N	S	S	2.54	ND
Heart	F	.23	1>4	ND	ND	S	2.49	S ⁻
			1>5	ND	ND	S	3.44	S ⁻
	M	.00	1>4	N	S	S	2.74	N
			1>5	S	S	S	4.50	S ⁻
			2>5	S	S	S	3.26	ND
			3>5	S	S	S	3.26	ND
			4>5	S	S	S	2.46	ND
	F	.11	1>2	ND	ND	S	2.12	N
			1>5	ND	ND	S	2.71	N

Appendix IV-19A
(cont'd) page 2

Parameter	Sex	ANOVA	Groups	Q	LSD	UAREP t-Test	t-Test Value	HLA Analysis
Heart/BW	F	.38	1>2	ND	ND	S	2.11	N
Liver	M	.02	1>2	N	S	N	-	N
			2<3	S	S	S	2.75	ND
			2<4	N	S	N	-	ND
			3<5	S	S	S	2.64	ND
	F	.17	1>4	ND	ND	S	2.37	N
			1>5	ND	ND	S	2.21	N
Liver/BW	M	.00	1>2	N	S	S	2.51	S ⁻
			1<3	N	S	N	-	N
			1<5	N	N	S	3.01	N
			2<3	S	S	S	3.34	ND
			2<4	S	S	S	3.79	ND
			2<5	S	S	S	4.05	ND
Kidney/BW	M	.00	1<3	N	S	S	2.20	S ⁺
			1<4	S	S	S	2.23	S ⁺
			1<5	S	S	S	4.80	S ⁺
			2<3	S	S	S	2.37	ND
			2<4	S	S	S	2.39	ND
			2<5	S	S	S	4.37	ND
Adrenal	F	.44	1>5	ND	ND	S	2.23	S ⁻
Testes/BW	M	.00	1<5	S	S	S	4.61	S ⁺
			2<5	S	S	S	5.52	ND
			3<5	S	S	S	4.46	ND
			4<5	S	S	S	4.74	ND

Appendix IV-19A
(cont'd) page 3

Group	Sex	Parameter	HLA Value	Type of Discrepancy	UAREP Value
4	F	Ovary ratio %	0.0464	C	0.0462
		Ovary ratio (SD)	±.0230	C	.0222
1	M	Sem. vesicles ratio%	0.243	R	0.244 (0.2435)
1	M	Prostate weight	0.91	R	0.90 (0.9057)
		Prostate weight (SD)	±0.41	R	0.40 (0.4053)
1	M	Prostate ratio %	0.18	C	0.17
		Prostate ratio (SD)	±0.06	C	0.07
5	F	Uterus weight	0.72	C	0.70
		Uterus ratio (SD)	±0.10	C	0.11

*In group 5 males, when queried, HLA was unable to tell UAREP which of 21 rats had its body weight deleted or why. Therefore UAREP used all 21 weights instead of 20 HLA reported. This "N" discrepancy of group 5 males resulted in 13 other discrepancies of the mean and 12 other discrepancies of standard deviations, which were noted but not recorded. The Necropsy Sheet on group 5 Female rat 84021 says "Thyroids missing at autopsy" and no weight was recorded. Therefore UAREP used an "N" of 9 instead of the 10 shown by HLA.

APPENDIX IV-20

COMPARISON OF UREP (U) AND EPL (E) DATA ON FREQUENCIES OF TYPES OF HISTOLOGICALLY
PROVEN TUMORS IN MALE AND FEMALE RATS RECEIVING ASPARTAME
OR SERVING AS CONTROLS

	Male Group										Female Group									
	1		2		3		4		5		1		2		3		4		5	
	U	E	U	E	U	E	U	E	U	E	U	E	U	E	U	E	U	E	U	E
Adrenal	56	59	20	20	19	18	50	49	39	38	59	60	33	33	28	29	40	40	40	40
Cortical adenoma	2	0	0	0	1	0	0	0	2	0	3	1	0	0	2	2	3	0	2	1
Pheochromocytoma	6	7	8	9	1	2	4	6	4	9	2	2	1	0	2	2	0	1 ^a	1	1
Ganglioneuroma	1	1	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0
Pituitary	58	56	13	13	16	18	39	38	39	39	59	60	26	26	34	34	38	40	37	39
Adenoma	11	16	1	2	7	7	8	9	7	8	30	29	16	18	17	20	16	20	16	18
Adenocarcinoma	2	1	1	0	0	0	2	0	0	0	4	5	3	2	2	1	4	1	2	0
Thyroid	51	57	10	12	7	11	39	35	35	40	57	59	12	12	13	12	33	37	38	38
Adenoma	1	1	0	0	0	0	0	0	0	1	0	1	0	1	2	2	0	0	0	0
C-cell carcinoma	1	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0
Parathyroid adenoma	0	0	1	1	0	0	1	2	0	0	0	0	0	0	0	0	0	0	0	0
Parathyroid adenocarcinoma	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Paraganglioma	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0
Liver	58	59	19	21	23	23	33	40	40	38	55	60	19	22	19	20	40	40	37	40
Neoplastic nodule/adenoma	1	1	0	0	0	0	4	0	0	1	5	2	2	0	3	0	1	0	2	0
Hepatocellular carcinoma	1	0	0	0	0	0	0	0	1	0	1	0	1	1	1	0	0	0	0	0
Bile duct carcinoma	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0
Kidney	59	59	27	28	29	30	35	40	38	40	56	60	28	32	23	25	39	40	35	40
Lipoma	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0
Lung	59	58	36	35	37	36	40	40	40	40	60	59	36	36	35	35	40	39	40	40
Alveolar adenoma	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0
Carcinoma	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	1
Pancreas	56	57	14	12	13	10	40	40	39	36	60	60	11	11	12	11	39	40	39	38
Islet cell tumor	1	1	1	1	0	0	2	2	4	1	0	0	0	0	1	1	0	0	0	0

Appendix IV-20
Continued, page 3

	Male Group										Female Group									
	1		2		3		4		5		1		2		3		4		5	
	U	E	U	E	U	E	U	E	U	E	U	E	U	E	U	E	U	E	U	E
All Organs																				
Lymphoma	6	6	2	2	2	0	0	0	1	2	1	2	0	0	1	4	3	1	0	1
Mesothelioma	0	0	0	0	0	0	2	2	0	0	0	0	0	0	0	1	0	0	0	0
Total tumors (UAREP count)	37	39	22	21	15	13	32	28	21	24	79	76	52	47	59	56	49	51	42	40
Original EPL Total	49		27		16		30		24		77		50		60		49		40	
Average number tumors per animal	UAREP .6		.5		.4		.8		.5		1.3		1.3		1.2		1.2		1.0	
	EPL .6		.5		.3		.7		.6		1.3		1.2		1.4		1.3		1.0	

EPL data as shown in Figure No. 5 page 45, E-34 was recomputed for comparative reasons stated below.

- a 83-936, 4F lists "Adrenal - pheochromocytoma" in Figure No. 6 page 63, E-34, whereas Figure No. 5 page 48 shows "Pheochromocytoma - 0" under Group 4 Female.
- b To compare the results obtained by UAREP and EPL, only one tumor of any specific type was counted per animal, even though EPL may have diagnosed two tumors of the same type. In addition, UAREP did not differentiate between "Adenoma" and "Fibroadenoma" in this table. In some instances, EPL diagnosed both Fibroadenoma and Adenoma on the mammary gland of a single animal; this appears in the UAREP table as 1 Fibroadenoma. The following animals account for this discrepancy: 83-791, 83-300, and 83-801 in group 2F
- c Animal 83-669, 1F, lists "Mammary - Adenocarcinoma" & "Mammary - Adenocarcinoma/scirrhous" This is counted as one tumor by UAREP. Animal 83-661, 3F lists "Mammary - Adenocarcinoma" and "Mammary - Adenocarcinoma/Papillary" This is counted as one tumor by UAREP.
- d Table No. 8, page 208, E-33 shows "Tissue mass - Fibroadenoma" for Group 4F animals 83-938, 83-941, and 83-961. These do not appear in Figure 6, page 63, E-34.

The figures opposite each organ show the number of rats with sections of the organ examined.

APPENDIX IV-21

RATS WITH TUMORS HISTOLOGICALLY PROVEN BY UAREP

WITH WEEK OF PRESUMED INITIAL

OBSERVATION OF TUMOR

<u>Group</u>	<u>Path Number</u>	<u>Animal Number</u>	<u>Tumor Type</u>	<u>Time</u>
1 Male	64-591	83-620	Lymphoma	104
	64-592	83-621	Adrenal - Pheochromocytoma	104
	64-593	83-622	Pituitary - Adenoma	104
	64-594	83-624	Adrenal - Pheochromocytoma	104
			Adrenal - Ganglioneuroma	104
			Pituitary - Adenocarcinoma	104
			Pancreas - Islet cell tumor	104
	64-597	83-627	Liver - Hepatocellular carcinoma	104
	64-598	83-631	Skin - Histiocytoma	104
	64-599	83-635	Adrenal - Cortical adenoma	104
			Liver - Neoplastic nodule	104
	64-600	83-640	Pituitary - Adenoma	104
			Thyroid - C-cell carcinoma	104
	64-601	83-646	Pituitary - Adenoma	104
	64-602	83-648	Pituitary - Adenoma	104
	64-603	83-651	Adrenal - Pheochromocytoma	104
			Pituitary - Adenoma	104
			Brain - Astrocytoma	104

UAREP recognizes that data on the time of onset of tumors is grossly approximate when using criteria either (a) of time of sacrifice or death with proven tumor not previously recognized or (b) first date of clinical observation of swelling subsequently confirmed histologically as a tumor. Better data is not available.

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continued, page two

<u>Group</u>	<u>Path Number</u>	<u>Animal Number</u>	<u>Tumor Type</u>	<u>Time</u>
1 Male	64-604	83-652	Testis - Interstitial cell tumor	104
	64-607	83-658	Pituitary - Adenocarcinoma	104
	64-609	83-662	Pituitary - Adenoma	104
	64-610	83-664	Thyroid - Adenoma	104
	64-611	83-606	Pituitary - Adenoma	84
	64-612	83-608	Lymphoma	35
	64-614	83-610	Lymphoma	36
	64-616	83-612	Pituitary - Adenoma	59
	64-617	83-614	Adrenal - Pheochromocytoma	102
	64-621	83-619	Pituitary - Adenoma	104
	64-625	83-630	Adrenal - Pheochromocytoma	103
			Pituitary - Adenoma	103
	64-630	83-637	Lymphoma	34
	64-632	83-639	Lymphoma	104
	64-633	83-641	Adrenal - Cortical adenoma	101
	64-634	83-642	Pituitary - Adenoma	76
	64-637	83-645	Lymphoma	57
	64-638	83-647	Skin - Giant cell tumor	84
	64-643	83-657	Adrenal - Pheochromocytoma	101
2 Male	64-769	83-731	Mammary gland - Adenocarcinoma	100
	64-771	83-737	Pituitary - Adenoma	104
			Pancreas - Islet cell tumor	104
			Testis - Interstitial cell tumor	104

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continued, page three

<u>Group</u>	<u>Path Number</u>	<u>Animal Number</u>	<u>Tumor Type</u>	<u>Time</u>
2 Male	64-774	83-744	Pituitary - Adenocarcinoma	104
	64-775	83-745	Adrenal - Pheochromocytoma	104
			Brain - Astrocytoma	104
	64-776	83-746	Adrenal - Pheochromocytoma	104
	64-777	83-750	Adrenal - Pheochromocytoma	104
			Thyroid - Parathyroid adenoma	104
	64-782	83-758	Adrenal - Pheochromocytoma	104
			Skin - Keratoacanthoma	104
	64-784	83-762	Adrenal - Pheochromocytoma	104
	64-787	83-728	Skin - Fibroma	64
	64-789	83-730	Adrenal - Pheochromocytoma	101
	64-790	83-733	Adrenal - Pheochromocytoma	104
	64-791	83-734	Lymphoma	70
	64-792	83-735	Prostate - Adenocarcinoma	82
	64-795	83-740	Adrenal - Pheochromocytoma	99
	64-800	83-749	Lymphoma	92
	64-804	83-759	Tissue mass - Epithelial neoplasm	84
	64-806	83-763	Tissue mass - Fibrosarcoma	92
3 Male	64-736	83-826	Pituitary - Adenoma	104
			Skin - Fibrosarcoma	104
			Lymphoma	104
	64-738	83-830	Adrenal - Pheochromocytoma	104

Appendix IV-21
continued, page four

<u>Group</u>	<u>Path Number</u>	<u>Animal Number</u>	<u>Tumor Type</u>	<u>Time</u>
3 Male	64-740	83-832	Pituitary - Adenoma	104
	64-742	83-834	Pituitary - Adenoma	104
	64-745	83-840	Pituitary - Adenoma	104
	64-753	83-815	Skin - Fibroma	64
	64-755	83-817	Pituitary - Adenoma	81
	64-758	83-820	Adrenal - Cortical adenoma	74
	64-759	83-821	Lymphoma	99
	64-763	83-835	Pituitary - Adenoma	76
	64-764	83-837	Brain - Astrocytoma	76
	64-765	83-839	Urinary bladder - Papilloma	71
	64-767	83-844	Pituitary - Adenoma	103
4 Male	64-688	83-887	Liver - Neoplastic nodule	104
	64-691	83-891	Pituitary - Adenocarcinoma	104
			Liver - Neoplastic nodule	104
	64-693	83-898	Pituitary - Adenoma	104
			Thyroid - Parathyroid adenoma	104
			Testis - Interstitial cell tumor	104
			Mesothelioma	104
	64-694	83-899	Mesothelioma	104
	64-695	83-901	Adrenal - Pheochromocytoma	104
			Pituitary - Adenoma	104
	64-696	83-903	Pituitary - Adenoma	104
	64-697	83-904	Pituitary - Adenoma	104

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continued, page five

<u>Group</u>	<u>Path Number</u>	<u>Animal Number</u>	<u>Tumor Type</u>	<u>Time</u>
4 Male	64-700	83-907	Adrenal - Pheochromocytoma	104
	64-701	83-908	Pancreas - Islet cell tumor	104
	64-704	83-912	Pituitary - Adenocarcinoma	104
	64-705	83-913	Pituitary - Adenoma	104
			Liver - Neoplastic nodule	104
	64-706	83-916	Pituitary - Adenoma	104
	64-707	83-919	Brain - Astrocytoma	104
			Skin - Keratoacanthoma	104
	64-709	83-923	Adrenal - Pheochromocytoma	104
			Thyroid - Parathyroid adenoma	104
	64-710	83-925	Adrenal - Pheochromocytoma	104
			Pancreas - Islet cell tumor	104
	64-712	83-888	Liver - Neoplastic nodule	59
			Brain - Oligodendroglioma	59
	64-713	83-892	Brain - Astrocytoma	49
			Tissue mass - Sarcoma	34
	64-715	83-895	Brain - Astrocytoma	100
	64-716	83-896	Tissue mass - Keratoacanthoma	100
	64-722	83-915	Pituitary - Adenoma	99
			Skin - Squamous cell carcinoma	99
	64-723	83-917	Pituitary - Adenoma	98
5 Male	64-648	83-966	Adrenal - Pheochromocytoma	104
			Adrenal - Cortical adenoma	104
			Pituitary - Adenoma	104
	64-649	83-970	Pituitary - Adenoma	104

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continued, page six

<u>Group</u>	<u>Path Number</u>	<u>Animal Number</u>	<u>Tumor Type</u>	<u>Time</u>
5 Male	64-655	83-983	Pituitary - Adenoma	104
	64-658	83-989	Liver - Hepatocellular carcinoma	104
	64-659	83-990	Pituitary - Adenoma	104
	64-661	83-994	Lymphoma	104
	64-662	83-996	Pituitary - Adenoma	104
	64-663	83-997	Pancreas - Islet cell tumor	104
	64-664	83-998	Adrenal - Pheochromocytoma	104
	64-665	83-999	Pancreas - Islet cell tumor	104
	64-667	84-002	Pituitary - Adenoma	104
			Pancreas - Islet cell tumor	104
	64-668	84-005	Pancreas - Islet cell tumor	104
	64-672	83-973	Adrenal - Pheochromocytoma	94
	64-675	83-976	Adrenal - Cortical adenoma	69
	64-679	83-986	Pituitary - Adenoma	99
	64-687	83-971	Adrenal - Pheochromocytoma	104
			Skin - Hemangioma	104
			Tissue mass - Lipoma	104
1 Female	64-808	83-667	Pituitary - Adenoma	104
	64-809	83-668	Pituitary - Adenoma	104
			Mammary gland - Adenocarcinoma	92
	64-810	83-669	Pituitary - Adenoma	104
			Mammary gland - Adenocarcinoma	100
			Liver - Hepatocellular carcinoma	104

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continued, page seven

<u>Group</u>	<u>Path Number</u>	<u>Animal Number</u>	<u>Tumor Type</u>	<u>Time</u>
1 Female	64-811	83-672	Pituitary - Adenocarcinoma	104
			Liver - Neoplastic nodule	104
			Mammary gland - Fibroadenoma	92
	64-812	83-674	Pituitary - Adenoma	104
			Mammary gland - Fibroadenoma	100
	64-813	83-677	Adrenal - Cortical adenoma	104
			Pituitary - Adenoma	104
	64-814	83-678	Liver - Neoplastic nodule	104
			Mammary gland - Fibroadenoma	76
	64-816	83-681	Pituitary - Adenoma	104
			Mammary gland - Fibroadenoma	84
	64-817	83-682	Pituitary - Adenoma	104
	64-818	83-684	Pituitary - Adenoma	104
			Mammary gland - Fibroadenoma	84
	64-819	83-685	Liver - Neoplastic nodule	88
			Mammary gland - Fibroadenoma	88
	64-820	83-688	Adrenal - Pheochromocytoma	104
			Pituitary - Adenocarcinoma	104
	64-821	83-689	Mammary gland - Fibroadenoma	100
	64-822	83-690	Thyroid - C-cell carcinoma	104
	64-823	83-692	Mammary gland - Adenocarcinoma	84
			Mammary gland - Fibroadenoma	84
	64-824	83-694	Adrenal - Pheochromocytoma	104
			Pituitary - Adenoma	104
	64-825	83-696	Pituitary - Adenoma	104

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continued, page eight

<u>Group</u>	<u>Path Number</u>	<u>Animal Number</u>	<u>Tumor Type</u>	<u>Time</u>
1 Female	64-826	83-709	Adrenal - Cortical adenoma	104
			Pituitary - Adenoma	104
			Liver - Neoplastic nodule	104
			Mammary gland - Fibroadenoma	104
	64-827	83-713	Pituitary - Adenoma	104
			Liver - Neoplastic nodule	104
			Mammary gland - Fibroadenoma	92
	64-829	83-717	Mammary gland - Fibroadenoma	104
	64-830	83-719	Pituitary - Adenoma	104
	64-831	83-721	Pituitary - Adenoma	104
	64-832	83-723	Pituitary - Adenoma	104
			Mammary gland - Fibroadenoma	104
			Mammary gland - Papillary adenoma	104
	64-833	83-724	Adrenal - Cortical adenoma	104
			Pituitary - Adenoma	104
	64-834	83-666	Pituitary - Adenoma	86
	64-835	83-670	Mammary gland - Fibroadenoma	60
	64-836	83-671	Tissue mass - Subcutaneous sarcoma	68
	64-837	83-673	Pituitary - Adenoma	103
			Ovary - Granulosa cell tumor	103
	64-840	83-680	Lymphoma	69
	64-841	83-683	Pituitary - Adenoma	74
	64-842	83-686	Pituitary - Adenoma	75
	64-844	83-691	Pituitary - Adenoma	95
			Mammary gland - Adenocarcinoma	56
	64-846	83-695	Mammary gland - Fibroadenoma	38

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continued, page nine

<u>Group</u>	<u>Path Number</u>	<u>Animal Number</u>	<u>Tumor Type</u>	<u>Time</u>
1 Female	64-847	83-697	Mammary gland - Fibroadenoma	36
			Urinary bladder - Papilloma	102
	64-848	83-698	Pituitary - Adenoma	94
			Mammary gland - Fibroadenoma	94
	64-850	83-700	Pituitary - Adenoma	89
			Mammary gland - Fibroadenoma	68
	64-851	83-701	Mammary gland - Fibroma	68
			Skin - Hemangioma	103
	64-853	83-703	Pituitary - Adenoma	86
			Mammary gland - Fibroadenoma	64
	64-855	83-705	Pituitary - Adenoma	100
	64-856	83-706	Pituitary - Adenocarcinoma	101
			Mammary gland - Fibroadenoma	100
	64-858	83-708	Pituitary - Adenoma	104
	64-860	83-711	Pituitary - Adenoma	102
	64-861	83-712	Pituitary - Adenoma	104
	64-862	83-714	Mammary gland - Fibroadenoma	60
	64-864	83-718	Pituitary - Adenoma	91
	64-865	83-720	Pituitary - Adenoma	68
			Mammary gland - Fibroadenoma	50
	64-866	83-722	Pituitary - Adenocarcinoma	91
			Mammary gland - Fibroadenoma	84
2 Female	64-989	83-769	Pituitary - Adenoma	104
			Brain - Astrocytoma	104
			Mammary gland - Fibroadenoma	92

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continued, page ten

<u>Group</u>	<u>Path Number</u>	<u>Animal Number</u>	<u>Tumor Type</u>	<u>Time</u>
2 Female	64-990	83-771	Pituitary - Adenoma	104
			Mammary gland - Fibroadenoma	104
			Mammary gland - Adenoma	104
	64-991	83-773	Uterus - Carcinoma	104
	64-992	83-779	Uterus - Polyp	104
			Liver - Neoplastic nodule	104
	64-993	83-780	Adrenal - Pheochromocytoma	104
			Mammary gland - Fibroadenoma	80
	64-994	83-781	Pituitary - Adenoma	104
	64-995	83-783	Pituitary - Adenoma	104
			Mammary gland - Fibroadenoma	104
	64-997	83-785	Pituitary - Adenoma	104
	64-998	83-786	Pituitary - Adenoma	104
			Mammary gland - Fibroadenoma	33
	64-999	83-787	Pituitary - Adenoma	104
	65-000	83-789	Pituitary - Adenocarcinoma	104
			Mammary gland - Fibroadenoma	88
	65-001	83-790	Pituitary - Adenocarcinoma	104
			Liver - Neoplastic nodule	104
			Mammary gland - Fibroadenoma	104
	65-002	83-791	Pituitary - Adenoma	104
			Mammary gland - Fibroadenoma	84
	65-003	83-792	Pituitary - Adenoma	104
			Mammary gland - Fibroadenoma	68
	65-004	83-793	Pituitary - Adenoma	104
			Mammary gland - Adenocarcinoma	104

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continued, page eleven

<u>Group</u>	<u>Path Number</u>	<u>Animal Number</u>	<u>Tumor Type</u>	<u>Time</u>
2 Female	65-005	83-794	Mammary gland - Fibroadenoma	104
	65-008	83-800	Pituitary - Adenoma	104
			Mammary gland - Fibroadenoma	84
			Mammary gland - Adenocarcinoma	84
	65-009	83-801	Pituitary - Adenoma	104
			Mammary gland - Fibroadenoma	76
	65-010	83-802	Pituitary - Adenoma	104
			Liver - Hepatocellular carcinoma	104
	65-011	83-766	Brain - Astrocytoma	69
	65-012	83-767	Pituitary - Adenocarcinoma	103
	65-013	83-770	Pituitary - Adenoma	87
	65-014	83-772	Mammary gland - Fibroadenoma	99
	65-015	83-774	Lung - Metastatic tumor	93
			Mammary gland - Fibroadenoma	93
	65-016	83-775	Tissue mass - Neurofibroma	33
	65-017	83-776	Mammary gland - Fibroadenoma	72
	65-019	83-778	Pituitary - Adenoma	86
	65-020	83-782	Skin - Keratoacanthoma	85
	65-022	83-796	Mammary gland - Fibroadenoma	76
	65-024	83-799	Mammary gland - Fibroadenoma	72
	65-026	83-804	Mammary gland - Fibroadenoma	72
	65-027	83-805	Pituitary - Adenoma	102
			Mammary gland - Fibroadenoma	84

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continued, page twelve

<u>Group</u>	<u>Path Number</u>	<u>Animal Number</u>	<u>Tumor Type</u>	<u>Time</u>
3 Female	64-949	83-847	Pituitary - Adenoma	104
	64-950	83-850	Thyroid - Adenoma	104
			Pancreas - Islet cell tumor	104
	64-951	83-854	Pituitary - Adenoma	104
			Mammary gland - Fibroadenoma	30
			Mammary gland - Adenocarcinoma	30
	64-952	83-855	Pituitary - Adenoma	104
			Liver - Neoplastic nodule	104
			Mammary gland - Fibroadenoma	104
	64-953	83-859	Mammary gland - Adenocarcinoma	100
			Mammary gland - Fibroadenoma	100
	64-954	83-864	Adrenal - Pheochromocytoma	104
			Pituitary - Adenoma	104
	64-955	83-865	Pituitary - Adenoma	104
			Thyroid - Adenoma	104
	64-956	83-868	Pituitary - Adenoma	104
			Skin - Fibroma	92
	64-957	83-870	Adrenal - Cortical adenoma	104
			Pituitary - Adenoma	104
			Mammary gland - Fibroadenoma	68
	64-958	83-872	Adrenal - Cortical adenoma	104
			Mammary gland - Fibroadenoma	84
	64-959	83-873	Mammary gland - Fibroadenoma	100
	64-960	83-874	Pituitary - Adenoma	104
	64-961	83-875	Liver - Neoplastic nodule	104
			Mammary gland - Fibroadenoma	56
			Ovary - Granulosa cell tumor	104

Appendix IV-21
continued, page thirteen

<u>Group</u>	<u>Path Number</u>	<u>Animal Number</u>	<u>Tumor Type</u>	<u>Time</u>
3 Female	64-962	83-876	Pituitary - Adenoma	104
			Mammary gland - Fibroadenoma	100
			Ovary - Granulosa cell tumor	104
	64-964	83-882	Pituitary - Adenoma	104
			Mammary gland - Fibroadenoma	104
	64-965	83-883	Adrenal - Pheochromocytoma	104
			Pituitary - Adenoma	104
			Liver - Neoplastic nodule	104
	64-966	83-884	Mammary gland - Fibroadenoma	104
	64-967	83-885	Mammary gland - Fibroadenoma	72
	64-970	83-851	Mammary gland - Fibroadenoma	56
	64-973	83-856	Pituitary - Adenocarcinoma	101
	64-974	83-857	Lymphoma	30
	64-975	83-858	Mammary gland - Fibroadenoma	34
	64-976	83-860	Pituitary - Adenoma	104
	64-977	83-861	Pituitary - Adenocarcinoma	102
			Liver - Hepatocellular Carcinoma	102
			Mammary gland - Adenocarcinoma	72
			Mammary gland - Fibroadenoma	72
	64-978	83-862	Pituitary - Adenoma	84
			Mammary gland - Adenocarcinoma	68
			Mammary gland - Fibroadenoma	68
	64-979	83-863	Pituitary - Adenoma	95
	64-980	83-866	Skin - Keratoacanthoma	92

Appendix IV-21
continued, page fourteen

<u>Group</u>	<u>Path Number</u>	<u>Animal Number</u>	<u>Tumor Type</u>	<u>Time</u>	
3 Female	64-981	83-867	Pituitary - Adenoma	104	
			Thyroid - Paraganglioma	104	
			Mammary gland - Fibroadenoma	104	
	64-983	83-871	Pituitary - Adenoma	81	
			Mammary gland - Fibroadenoma	81	
	64-985	83-878	Mammary gland - Fibroadenoma	56	
	64-987	83-880	Pituitary - Adenoma	51	
			Mammary gland - Adenocarcinoma	48	
	4 Female	64-908	83-927	Pituitary - Adenocarcinoma	104
64-909		83-931	Pituitary - Adenoma	104	
64-912		83-938	Pituitary - Adenoma	104	
			Mammary gland - Fibroadenoma	92	
64-913		83-941	Mammary gland - Fibroadenoma	100	
			Ovary - Granulosa cell tumor	104	
			Lymphoma	104	
64-915		83-947	Pituitary - Adenocarcinoma	104	
			Liver - Neoplastic nodule	104	
64-916		83-949	Mammary gland - Fibroadenoma	104	
64-918		83-952	Pituitary - Adenocarcinoma	104	
			Mammary gland - Adenocarcinoma	100	
64-920		83-958	Pituitary - Adenoma	104	
			Mammary gland - Fibroadenoma	64	
64-921	83-961	Pituitary - Adenoma	104		
		Mammary gland - Fibroadenoma	64		

Appendix IV-21
continued, page fifteen

<u>Group</u>	<u>Path Number</u>	<u>Animal Number</u>	<u>Tumor Type</u>	<u>Time</u>
4 Female	64-922	83-926	Pituitary - Adenoma	98
			Mammary gland - Fibroadenoma	80
			Mammary gland - Papillary adenoma	80
			Lymphoma	98
	64-923	83-928	Pituitary - Adenoma	73
			Mammary gland - Fibroadenoma	64
	64-925	83-930	Pituitary - Adenoma	84
	64-926	83-934	Pituitary - Adenocarcinoma	85
			Brain - Astrocytoma	85
	64-928	83-936	Adrenal - Cortical adenoma	97
			Pituitary - Adenoma	97
			Mammary gland - Adenocarcinoma	50
			Mammary gland - Fibroadenoma	50
	64-930	83-939	Pituitary - Adenoma	103
			Mammary gland - Fibroadenoma	64
	64-931	83-940	Mammary gland - Fibroadenoma	60
			Skin - Angiofibroma	60
	64-932	83-943	Adrenal - Cortical adenoma	82
			Pituitary - Adenoma	82
	64-933	83-944	Pituitary - Adenoma	98
			Mammary gland - Adenocarcinoma	80
	64-936	83-948	Pituitary - Adenoma	100
	64-940	83-956	Pituitary - Adenoma	80
			Mammary gland - Fibroadenoma	52
	64-941	83-957	Mammary gland - Adenocarcinoma	68
	64-943	83-960	Mammary gland - Fibroadenoma	56

Appendix IV-21
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<u>Group</u>	<u>Path Number</u>	<u>Animal Number</u>	<u>Tumor Type</u>	<u>Time</u>
4 Female	64-944	83-962	Pituitary - Adenoma	91
	64-945	83-963	Adrenal - Cortical adenoma	96
			Pituitary - Adenoma	96
	64-946	83-964	Pituitary - Adenoma	100
			Mammary gland - Adenocarcinoma	64
	64-947	83-965	Mammary gland - Fibroadenoma	42
			Lymphoma	82
5 Female	64-869	84-016	Adrenal - Cortical adenoma	104
			Adrenal - Pheochromocytoma	104
			Pituitary - Adenocarcinoma	104
	64-870	84-017	Pituitary - Adenocarcinoma	104
	64-871	84-021	Pituitary - Adenoma	104
			Uterus - Benign vascular tumor or leiomyoma	104
	64-873	84-036	Pituitary - Adenoma	104
	64-874	84-037	Pituitary - Adenoma	104
			Mammary gland - Fibroadenoma	88
	64-876	84-043	Adrenal - Cortical adenoma	104
			Pituitary - Adenoma	104
	64-877	84-045	Pituitary - Adenoma	104
			Mammary gland - Fibroadenoma	88
	64-879	84-007	Pituitary - Adenoma	89
			Mammary gland - Fibroadenoma	80
	64-880	84-008	Pituitary - Adenoma	79
			Mammary gland - Adenocarcinoma	60
	64-881	84-010	Brain - Medulloblastoma/meningeal sarcoma	13

Appendix IV-21
continued, page seventeen

<u>Group</u>	<u>Path Number</u>	<u>Animal Number</u>	<u>Tumor Type</u>	<u>Time</u>
5 Female	64-882	84-011	Mammary gland - Fibroadenoma	38
	64-883	84-012	Pituitary - Adenoma	100
			Mammary gland - Fibroadenoma	76
	64-885	84-014	Mammary gland - Fibroadenoma	42
	64-886	84-015	Pituitary - Adenoma	99
	64-888	84-019	Pituitary - Adenoma	67
			Brain - Astrocytoma	67
	64-889	84-020	Pituitary - Adenoma	102
			Mammary gland - Adenocarcinoma	80
	64-891	84-023	Pituitary - Adenoma	101
	64-892	84-025	Mammary gland - Fibroadenoma	68
	64-893	84-026	Pituitary - Adenoma	90
			Mammary gland - Adenocarcinoma	72
	64-896	84-029	Mammary gland - Adenocarcinoma	30
	64-897	84-030	Lung - Adenocarcinoma	101
			Mammary gland - Fibroadenoma	72
			Mammary gland - Adenocarcinoma	72
	64-900	84-033	Pituitary - Adenoma	91
	64-901	84-034	Pituitary - Adenoma	100
	64-906	84-042	Liver - Neoplastic nodule	80
			Mammary gland - Fibroadenoma	38
	64-907	84-044	Pituitary - Adenoma	94
			Mammary gland - Duct papilloma	88
			Liver - Neoplastic nodule	94

APPENDIX IV-22
LISTING OF SECTIONS WITH TUMOR DIAGNOSES
BY EPL AND MISSING BY UAREP

<u>Group</u>	<u>Path Number</u>	<u>Animal Number</u>	<u>Organ</u>	<u>UAREP</u>	<u>EPL</u>
2M	64-788	83-729	Spleen	No section	Reticulum cell sarcoma
2M	64-799	83-748	Kidney	No section	Metastatic neoplasm
3M	64-736	83-826	Mammary gland	No section	Fibroadenoma
3M	64-758	83-820	Pituitary	No section	Adenoma
2F	65-013	83-770	Mammary gland	No section	Fibroadenoma
3F	64-971	83-852	Liver	No section	Reticulum cell sarcoma
3F	64-975	83-858	Bone marrow	No section	Reticulum cell sarcoma

APPENDIX IV-23

NUMBERS OF MALE AND FEMALE RATS WITH HISTOLOGICALLY PROVEN TUMORS AS DIAGNOSED BY UAREP (U) AND EPL (E)

	Male Groups									
	1		2		3		4		5	
	U	E	U	E	U	E	U	E	U	E
Any tumor	29	28	17	19	13	11	21	19	16	18
All malignant tumors	11	9	7	7	3	3	9	5	2	2
Benign tumors	21	19	11	12	11	8	17	14	14	16
Adrenal cortical tumors	2	-	0	-	1	-	0	-	2	-
Adrenal medullary tumors	6	-	8	-	1	-	4	-	4	-
Pituitary tumors	13	-	2	-	7	-	10	-	7	-
All mammary tumors	0	1	1	2	0	1	0	0	0	0
Malignant mammary tumors	0	0	1	1	0	0	0	0	0	0

	Female Groups									
	1		2		3		4		5	
	U	E	U	E	U	E	U	E	U	E
Any tumor	47	49	32	30	31	33	26	27	25	26
All malignant tumors	11	13	10	7	7	14	11	9	9	9
Benign tumors	44	36	29	23	29	19	22	18	22	17
Adrenal cortical tumors	3	-	0	-	2	-	3	-	2	-
Adrenal medullary tumors	2	-	1	-	2	-	0	-	1	-
Pituitary tumors	34	-	19	-	19	-	20	-	18	-
All mammary tumors	26	26	20	19	19	16	17	16	14	12
Malignant mammary tumors	4	7	2	2	5	6	5	7	5	7

EPL figures are taken from Figure No. 7, page 67, E-34, which did not contain data on Adrenal or Pituitary tumors. UAREP figures are based on tumors listed in Appendix IV-20 and IV-21. HLA figures for benign tumors are lower than UAREP's because they are based on animals with only a benign tumor whereas UAREP figures include animals with both a benign and malignant tumor. UAREP's sum of "benign" and "all malignant tumors" therefore exceeds "any tumor".

APPENDIX IV-24

COMPARISON OF COMPUTATIONS BY UAREP AND HLA OF PROBABILITIES OF TUMOR
INCIDENCE IN MALE AND FEMALE RATS RECEIVING ASPARTAME
OR SERVING AS CONTROLS

Group	Males				Significance P<0.05 HLA UAREP	Females				Significance P<0.05 HLA UAREP
	HLA P	UAREP P	HLA [N]	UAREP [N]		HLA P	UAREP P	HLA [N]	UAREP [N]	
Any Tumor										
1	79.6	86.7	35.2	33.4	1vs3	95.4	95.4	51.4	49.3	
2	64.1	74.0	29.6	22.9		87.7	93.4	34.2	34.3	
3	38.4	56.6	28.6	24.7		96.8	96.5	34.1	32.1	
4	64.4	74.8	29.5	28.1		85.7	87.6	31.5	29.7	
5	68.2	75.0	25.4	21.3		85.0	92.0	30.6	27.2	
Mammary Tumor										
1	4.1	0	24.4	0		55.0	59.7	47.3	43.6	
2	8.6	4.4	23.3	22.7		58.0	70.0	32.8	28.6	
3	4.7	0	21.3	0		50.6	67.3	31.6	28.2	
4	0	0	-	0		55.1	60.4	29.0	28.1	
5	0	0	-	0		40.1	43.8	29.9	32.0	
Mammary Carcinoma										
1	0	0	-	0		17.2	8.9	40.7	44.9	
2	4.5	4.4	22.2	22.7		7.6	15.3	26.3	19.6	
3	0	0	-	0		18.7	14.6	32.1	34.2	
4	0	0	-	0		22.5	16.4	31.1	30.5	
5	0	0	-	0		23.5	15.2	29.8	32.9	
Benign Tumor										
1	61.2	78.4	31.0	26.8	1vs3	75.1	93.9	47.9	46.9	
2	51.1	61.7	23.5	17.8		70.3	89.1	32.7	32.6	
3	29.9	53.4	26.8	22.5		70.8	94.3	26.8	30.8	
4	51.0	67.7	27.5	25.1		68.9	80.4	26.1	27.4	
5	63.3	68.2	25.3	20.5		70.0	88.6	24.3	24.8	
Any Malignant Tumor										
1	26.5	43.3	33.9	25.4	1vs5	33.4	33.7	38.9	32.6	
2	23.1	33.9	30.3	20.6		25.6	52.0	27.3	19.2	
3	11.1	21.2	27.0	18.9		42.8	24.3	32.7	28.8	
4	17.7	37.2	28.2	24.2		31.7	52.6	28.4	20.9	
5	7.1	16.0	28.2	12.5		31.8	42.7	28.3	21.1	
Adrenal Medulla										
1	34.3		17.5		2>3 P=.03	10.8		18.5		
2	53.9		14.8			7.7		13.0		
3	8.0		-12.5			15.4		13.0		
4	24.2		16.5			0		0		
5	26.0		15.4			13.3		7.5		

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(cont'd) page 2

(cont'd) page 2		Males				Females				
Group	HLA	UAREP	HLA	UAREP	Significance	HLA	UAREP	HLA	UAREP	Significance
	P	P	[N]	[N]	P<0.05		P		P	[N]
Pituitary Tumors										
1		55.0		23.6			83.9		40.5	
2		18.2		11.0	1>2		82.1		23.2	
3		38.8		18.0			79.8		23.8	
4		46.7		21.4			82.8		24.2	
5		43.6		16.1			85.9		21.0	
Adrenal Cortex										
1		12.9		15.5			15.8		19.0	
2		0		0			0			
3		3.2		31.2			15.4		13.0	
4		0		0			13.1		22.9	
5		10.8		18.5			25.0		8.0	

HLA data is from Figures No. 8, page 68 of E-34; UAREP data is based on Appendix IV-23.

P=calculated probability, X100, of developing a tumor during the total test period.

[N]=estimate of "effective number" of animals on test over the entire period which is number of tumor bearing rats/P

Only group comparisons which are statistically significant are shown under the appropriate columns.

APPENDIX IV-25A

COMPARISON OF SUMMARIES OF UAREP AND EPL HISTOPATHOLOGIC DIAGNOSES
ON NON-NEOPLASTIC CHANGES FOR MALE RATS TREATED WITH ASPARTAME FOR
104 WEEKS (E-34, FIGURE 9, PAGES 70-83)

[illegible]

Appendix IV-25A
(cont'd) page 2

	Group 1		Group 2		Group 3		Group 4		Group 5	
	UAREP	EPL	UAREP	EPL	UAREP	EPL	UAREP	EPL	UAREP	EPL
<u>Liver</u>	22	23	10	12	15	15	17	23	21	20
Bile duct Proliferation	5	16	2	6	6	7	5	19	3	11
Lymphoid infiltrate	14	17	7	10	13	9	17	20	16	15
Hematopoiesis	0	0	0	0	0	0	0	0	0	0
Hepatocyte vacuolation	2	0	1	0	0	0	0	0	4	3
Necrosis/ infarct	4	3	2	3	0	3	2	2	0	0
Fibrosis	2	1	1	1	3	2	2	2	2	0
Hepatitis	0	0	0	0	0	0	1	0	0	0
Abscess	0	0	0	0	0	0	0	1	0	0
Hyperplastic nodule/focus of change	2	3	0	1	0	1	1	6	0	0
<u>Kidney</u>	23	23	13	12	17	16	19	23	19	21
Interstitial nephritis	15	18	9	7	14	13	15	18	16	15
Glomerulo- sclerosis	11	7	9	0	11	0	14	0	16	7
Tubular dilatation	16	13	11	11	16	14	18	22	19	17
Tubular degeneration	0	0	0	0	0	0	0	0	0	11
Cortical cysts	11	1	2	0	0	0	1	0	4	1
Abscess	0	0	1	1	1	1	0	1	2	0
Pyelitis/ pyelonephritis	1	3	3	0	1	0	2	2	2	0

Appendix IV-25A
(cont'd) page 3

	<u>Group 1</u>		<u>Group 2</u>		<u>Group 3</u>		<u>Group 4</u>		<u>Group 5</u>	
	UAREP	EPL	UAREP	EPL	UAREP	EPL	UAREP	EPL	UAREP	EPL
(Kidney cont'd)										
Pelvic epithelial hyperplasia	0	1	3	0	7	2	2	4	6	5
Pelvic dilatation	0	0	0	0	0	0	0	0	0	0
<u>Lung</u>	23	23	14	14	19	18	23	23	21	21
Acute inflammation	20	15	14	2	17	3	22	1	17	10
Abscess	0	6	2	10	0	10	1	13	0	7
Chronic inflammation	23	21	14	12	19	16	23	23	19	17
Granulomatous inflammation	0	0	0	0	0	0	0	0	0	0
Congestion/ edema	2	0	2	0	6	0	6	1	0	0
Hemorrhage	1	0	1	0	1	0	0	0	0	0
Pneumocyte hyperplasia/ Alveolar macrophages	14	8	8	1	3	4	6	11	9	10
Fibrosis	0	0	0	0	0	0	0	0	0	0
<u>Heart</u>	23	22	10	10	10	10	23	23	19	17
Focal fibrosis	5	14	4	10	4	10	6	23	6	14
Mineralization	2	0	0	0	0	2	1	3	1	1
Focal myocarditis	0	0	0	0	4	0	0	0	1	1
Peri/Endocarditis	0	0	0	0	1	0	0	0	0	0
Endocarditis	0	0	0	0	0	0	0	0	0	0
Periarteritis	0	0	0	0	0	0	1	0	0	0

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(cont'd) page 4

	<u>Group 1</u>		<u>Group 2</u>		<u>Group 3</u>		<u>Group 4</u>		<u>Group 5</u>	
	UAREP	EPL	UAREP	EPL	UAREP	EPL	UAREP	EPL	UAREP	EPL
<u>Spleen</u>	23	23	10	9	12	10	23	23	20	21
Extramedullary hematopoiesis	1	22	1	9	0	8	2	23	0	21
Pigment	17	22	10	8	10	8	16	19	20	21
Reticuloendo- thelial cell hyperplasia	18	0	9	0	6	0	14	1	19	0
Lymphoid hyperplasia	5	0	1	0	1	0	7	0	4	0
<u>Skeletal Muscle</u>	16	23	0	0	0	0	0	0	21	21
Fibrosis	0	0	0	0	0	0	0	0	0	1
Myositis	0	0	0	0	0	0	0	0	0	0
<u>Peripheral Nerve</u>	17	23	0	0	0	0	1	0	21	21
Unremarkable	17	23	0	0	0	0	0	0	11	21
<u>Pancreas</u>	23	23	10	10	11	10	23	23	20	21
Fat necrosis	0	0	0	0	0	0	0	0	1	0
Fibrosis	0	7	0	3	4	5	0	9	6	12
Atrophy	0	4	0	2	0	6	0	8	0	8
Arteritis	1	3	0	0	2	3	4	4	1	1
Acute pancreatitis	0	3	0	0	3	1	4	3	0	1

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(cont'd) page 5

	<u>Group 1</u>		<u>Group 2</u>		<u>Group 3</u>		<u>Group 4</u>		<u>Group 5</u>	
	UAREP	EPL	UAREP	EPL	UAREP	EPL	UAREP	EPL	UAREP	EPL
<u>Stomach</u>	23	23	11	11	12	12	23	23	20	21
Ulcer	0	0	0	0	1	1	0	0	0	0
Gastritis	0	0	2	1	1	2	2	2	0	0
<u>Small Intestine</u>	23	23	9	10	10	10	22	22	20	21
Unremarkable	23	23	8	10	8	10	15	22	11	21
Hemorrhage	0	0	0	0	0	0	0	0	0	0
<u>Large Intestine</u>	23	23	10	10	10	9	22	22	19	20
Nematodiasis	7	5	2	1	1	1	0	1	3	3
Edema	0	0	0	0	0	0	0	0	0	0
Hemorrhage	0	1	0	0	0	0	0	0	0	0
<u>Eye</u>	23	23	0	0	0	0	0	0	21	20
Dacryoadenitis	2	12	0	0	0	0	0	0	1	6
Corneal vas- cularization	0	0	0	0	0	0	0	0	0	1
Inflammation	2	0	0	0	0	0	0	0	0	1
Loss of nuclear layer	1	1	0	0	0	0	0	0	0	0

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(cont'd) page 6

	<u>Group 1</u>		<u>Group 2</u>		<u>Group 3</u>		<u>Group 4</u>		<u>Group 5</u>	
	UAREP	EPL	UAREP	EPL	UAREP	EPL	UAREP	EPL	UAREP	EPL
<u>Lymph Node</u>	23	22	2	2	2	0	23	22	19	21
Adenitis	0	0	0	0	0	0	1	0	0	0
Necrosis	0	0	0	0	0	0	0	0	0	0
Reticuloendo- thelial cell proliferation	4	2	1	2	0	0	2	2	4	1
Lymphoid hyperplasia	7	0	1	0	0	0	2	0	7	1
<u>Salivary Gland</u>	23	23	2	2	2	1	23	23	20	21
Adenitis	0	4	0	0	0	0	0	2	0	0
<u>Brain</u>	23	23	16	0	21	1	23	1	20	21
Ventricle dilatation	3	1	0	0	0	0	0	0	0	0
Ventral compression	0	0	0	0	0	0	0	0	0	0
Glial prolif- eration	1	0	1	0	0	0	1	0	0	0
Encephalitis/ abscess	0	0	0	0	0	0	0	0	1	0
Meningitis	0	0	0	0	0	0	0	0	1	0
<u>Spinal Cord</u>	23	23	0	0	0	0	0	0	20	21
Unremarkable	22	23	0	0	0	0	0	0	6	21

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(cont'd) page 7

	Group 1		Group 2		Group 3		Group 4		Group 5	
	UAREP	EPL	UAREP	EPL	UAREP	EPL	UAREP	EPL	UAREP	EPL
<u>Mammary Gland</u>	10	10	1	1	0	2	0	0	5	10
Dilated ducts/ acini	2	1	0	0	0	1	0	0	0	0
Secretion	1	1	0	0	0	1	0	0	0	0
Mastitis	0	0	0	0	0	0	0	0	0	0
Periductal fibrosis	2	0	0	0	0	0	0	0	1	0
<u>Bone</u>	23	0	0	0	0	0	0	1	21	0
Osteomalacia	0	0	0	0	0	0	0	0	0	0
<u>Bone Marrow</u>	23	23	0	0	0	0	0	0	21	21
Hematogenic activity	16	23	0	0	0	0	0	0	1	21
<u>Testis</u>	23	23	11	10	13	13	19	23	20	21
Level of activity	17	22	11	10	6	13	8	22	20	21
Vacuolation/ epididymis	10	3	0	0	0	0	3	3	5	1
Arteritis	4	3	0	0	1	4	3	5	0	2
Epididymitis/ Orchitis	2	1	0	0	1	0	1	0	0	0
Hemorrhage	0	0	0	0	0	0	1	1	0	0
Mineralization	2	0	1	0	0	0	0	0	0	0

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(cont'd) page 8

	<u>Group 1</u>		<u>Group 2</u>		<u>Group 3</u>		<u>Group 4</u>		<u>Group 5</u>	
	UAREP	EPL	UAREP	EPL	UAREP	EPL	UAREP	EPL	UAREP	EPL
<u>Seminal Vesicle</u>	19	23	12	10	6	11	22	23	20	21
Vesiculitis	1	1	0	0	0	1	0	1	1	1
Atrophy	0	2	0	0	0	1	0	4	0	6
<u>Prostate</u>	22	23	11	11	11	10	23	23	20	21
Atrophy	12	1	0	0	0	1	0	0	1	1
Prostatitis acute	3	2	2	4	7	5	12	7	7	6
Prostatitis chronic	2	1	3	0	0	0	3	2	2	3
<u>Urinary Bladder</u>	23	23	16	18	20	21	20	23	21	21
Cystitis	1	3	0	0	1	5	2	6	2	2
Calculi/mucus plug	0	0	2	0	1	1	1	0	0	0
Epithelial hyperplasia	0	0	0	0	0	0	0	0	0	1
Squamous meta- plasia	0	0	1	0	0	0	1	0	0	0
<u>Skin</u>	4	4	2	1	4	3	6	6	11	1
Cellulitis/ dermatitis	2	4	1	1	2	3	6	6	1	1
Ulceration	2	4	0	0	0	2	0	5	0	0
Epidermal in- clusion cyst	1	0	0	1	1	0	0	1	3	0

The numbers opposite each organ show total number of sections examined

APPENDIX IV-25B

COMPARISON OF SUMMARIES OF UAREP AND EPL HISTOPATHOLOGIC DIAGNOSES
ON NON-NEOPLASTIC CHANGES FOR FEMALE RATS TREATED WITH ASPARTAME
FOR 104 WEEKS (E-34, FIGURE 9, PAGES 70-83)

[illegible]

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(cont'd) page 2

	<u>Group 1</u>		<u>Group 2</u>		<u>Group 3</u>		<u>Group 4</u>		<u>Group 5</u>	
	UAREP	EPL	UAREP	EPL	UAREP	EPL	UAREP	EPL	UAREP	EPL
<u>Liver</u>	22	26	12	16	13	15	14	14	8	10
Bile duct proliferation	10	22	6	9	3	4	6	7	5	9
Lymphoid infiltrate	17	22	6	9	8	6	12	13	8	8
Hematopoiesis	0	0	0	0	0	0	0	1	2	0
Hepatocyte vacuolation	2	1	0	0	1	2	0	0	3	0
Necrosis/infarct	2	2	0	1	2	2	1	1	0	0
Fibrosis	4	2	1	0	1	0	2	0	2	0
Hepatitis	0	0	0	0	0	0	0	0	0	0
Abscess	0	0	0	0	0	0	0	0	0	0
Hyperplastic nodule/focus of change	1	4	1	3	0	2	2	0	0	3
<u>Kidney</u>	22	26	15	19	12	14	14	14	8	10
Interstitial nephritis	10	9	8	0	9	3	5	1	6	2
Glomerulosclerosis	12	1	6	0	7	0	5	0	6	0
Tubular dilatation	17	7	8	4	9	5	7	5	5	0
Tubular degeneration	1	1	0	1	0	0	0	1	0	0
Cortical cysts	3	0	2	0	0	0	0	0	3	2
Abscess	0	0	1	0	0	0	0	0	0	0
Pyelitis/Pyelonephritis	3	0	1	1	1	1	0	0	0	0

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(cont'd) page 3

	Group 1		Group 2		Group 3		Group 4		Group 5	
	UAREP	EPL	UAREP	EPL	UAREP	EPL	UAREP	EPL	UAREP	EPL
(Kidney cont'd)										
Pelvic epithelial hyperplasia	5	0	5	2	1	1	0	0	2	1
Pelvic dilatation	1	1	0	0	0	0	0	0	0	0
<u>Lung</u>	26	25	19	19	18	18	14	13	10	10
Acute inflammation	20	7	16	1	16	1	10	0	10	0
Abscess	4	13	8	12	1	7	0	5	0	7
Chronic inflammation	26	24	19	11	18	14	14	11	10	10
Granulomatous inflammation	0	0	0	0	0	0	0	0	0	0
Congestion/edema	7	0	6	1	4	1	5	2	5	0
Hemorrhage	0	0	0	0	0	0	0	7	2	0
Pneumocyte hyperplasia/ Alveolar macrophages	14	10	6	1	7	8	3	0	5	7
Fibrosis	0	0	2	0	0	0	0	0	0	0
<u>Heart</u>	25	26	11	10	10	10	14	14	10	10
Focal fibrosis	6	23	6	10	1	10	3	14	1	8
Mineralization	1	0	0	0	0	0	0	0	0	0
Focal myocarditis	4	0	0	0	4	0	5	0	3	2
Peri/Epicarditis	0	0	1	0	0	0	0	0	1	0
Endocarditis	0	0	0	0	0	0	0	0	1	0
Periarteritis	2	1	0	0	0	0	0	0	0	0

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(cont'd) page 4

	<u>Group 1</u>		<u>Group 2</u>		<u>Group 3</u>		<u>Group 4</u>		<u>Group 5</u>	
	UAREP	EPL	UAREP	EPL	UAREP	EPL	UAREP	EPL	UAREP	EPL
<u>Spleen</u>	26	25	11	11	11	11	14	14	10	10
Extramedullary hematopoiesis	4	25	1	11	1	11	0	12	0	10
Pigment	26	25	11	11	10	10	14	14	10	10
Reticuloendo- thelial cell hyperplasia	16	0	3	0	10	0	5	0	4	0
Lymphoid hyperplasia	2	0	1	0	0	0	0	0	0	0
<u>Skeletal Muscle</u>	26	25	0	0	0	0	0	0	10	10
Fibrosis	0	0	0	0	0	0	0	0	0	0
Myositis	1	1	0	0	0	0	0	0	0	0
<u>Peripheral Nerve</u>	25	25	0	0	0	0	0	0	10	10
Unremarkable	21	25	0	0	0	0	0	0	6	10
<u>Pancreas</u>	26	26	11	11	10	10	13	14	10	10
Fat necrosis	0	0	0	0	0	0	0	0	0	0
Fibrosis	4	7	1	2	0	1	2	7	3	7
Atrophy	1	6	0	3	0	2	0	6	0	5
Arteritis	1	7	0	0	0	1	0	0	0	0
Acute pancreatitis	1	3	0	0	1	0	1	1	3	4

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(cont'd) page 5[illegible]

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(cont'd) page 6

	<u>Group 1</u>		<u>Group 2</u>		<u>Group 3</u>		<u>Group 4</u>		<u>Group 5</u>	
	UAREP	EPL	UAREP	EPL	UAREP	EPL	UAREP	EPL	UAREP	EPL
<u>Lymph Node</u>	25	25	6	4	3	1	14	14	10	10
Adenitis	0	0	0	0	0	0	0	0	0	0
Necrosis	0	0	0	0	0	0	0	0	0	0
Reticuloendo- thelial cell proliferation	7	4	1	1	1	1	4	3	5	1
Lymphoid hyperplasia	9	4	3	0	1	0	3	1	4	0
<u>Salivary Gland</u>	26	25	2	2	1	1	14	14	9	10
Adenitis	1	0	0	0	0	0	0	0	0	0
<u>Brain</u>	26	26	23	3	20	1	14	0	10	10
Ventricle dilatation	2	1	2	1	1	0	1	0	0	0
Ventral compression	2	0	6	3	4	1	2	0	2	0
Glial prolif- eration	0	0	0	0	0	0	0	0	0	0
Encephalitis/ abscess	0	0	0	0	0	0	0	0	0	0
Meningitis	0	0	0	0	0	0	0	0	0	0
<u>Spinal Cord</u>	20	26	0	0	0	0	0	0	9	10
Unremarkable	15	26	0	0	0	0	0	0	5	10
<u>Mammary Gland</u>	25	26	13	13	12	10	13	12	10	10
Dilated ducts/ acini	10	7	2	3	2	1	2	4	3	4
Secretion	9	9	2	3	2	1	0	3	4	4

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(cont'd) page 7

	<u>Group 1</u>		<u>Group 2</u>		<u>Group 3</u>		<u>Group 4</u>		<u>Group 5</u>	
	UAREP	EPL	UAREP	EPL	UAREP	EPL	UAREP	EPL	UAREP	EPL
(Mammary Gland continued)										
Mastitis	1	2	0	0	0	0	1	0	0	0
Periductal fibrosis	2	0	0	0	0	0	0	0	0	1
<u>Bone</u>	17	0	0	0	0	0	1	0	4	0
Osteomalacia	0	0	0	0	0	0	0	0	0	0
<u>Bone Marrow</u>	23	26	0	0	0	0	1	1	10	10
Hematogenic activity	2	26	0	0	0	0	0	1	0	10
<u>Urinary Bladder</u>	26	26	23	23	20	19	12	13	10	10
Cystitis	2	7	1	4	4	5	0	0	0	0
Calculi/mucus plug	0	0	0	0	0	0	0	0	0	0
Epithelial hyperplasia	0	0	1	0	0	0	1	0	0	0
Squamous metaplasia	0	0	0	0	0	0	0	0	0	0
<u>Ovary</u>	26	22	15	15	15	11	14	14	10	9
Oophoritis	1	2	0	2	0	0	1	2	0	0
Cystic follicle/ Paraovarian cyst	3	5	7	7	3	6	2	5	1	2
Salpingitis	1	1	3	0	0	0	1	0	0	0

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	<u>Group 1</u>		<u>Group 2</u>		<u>Group 3</u>		<u>Group 4</u>		<u>Group 5</u>	
	<u>UAREP</u>	<u>EPL</u>	<u>UAREP</u>	<u>EPL</u>	<u>UAREP</u>	<u>EPL</u>	<u>UAREP</u>	<u>EPL</u>	<u>UAREP</u>	<u>EPL</u>
<u>Uterus</u>	21	24	15	14	14	12	14	14	10	10
Endometritis	11	5	7	6	7	6	7	4	8	1
Epithelial hyperplasia	1	1	5	3	3	1	4	2	2	0
Squamous metaplasia	2	1	0	0	1	1	2	2	0	1
Endometrial polyp	0	0	1	1	0	0	0	0	0	0
<u>Skin</u>	17	2	0	0	3	0	9	0	10	0
Cellulitis/ dermatitis	1	2	0	0	0	0	0	0	0	0
Ulceration	1	1	0	0	0	0	0	0	0	0
Epidermal in- clusion cyst	0	0	0	0	0	0	0	0	0	0

The numbers opposite each organ show total number of sections examined

APPENDIX IV-25C

COMPARISON OF SUMMARIES OF UAREP AND EPL HISTOPATHOLOGIC DIAGNOSES ON
NON-NEOPLASTIC CHANGES FOR MALE RATS TREATED WITH ASPARTAME WHICH WERE
FOUND DEAD OR SACRIFICED IN A MORIBUND CONDITION

(E-34, FIGURE 9A, PAGES 84-97)

	<u>Group 1</u>		<u>Group 2</u>		<u>Group 3</u>		<u>Group 4</u>		<u>Group 5</u>	
	<u>UAREP</u>	<u>EPL</u>	<u>UAREP</u>	<u>EPL</u>	<u>UAREP</u>	<u>EPL</u>	<u>UAREP</u>	<u>EPL</u>	<u>UAREP</u>	<u>EPL</u>
<u>Adrenal</u>	33	36	7	7	5	5	17	16	19	18
Nodular hyperplasia	4	1	0	0	2	3	4	1	3	2
Vacuolation cortex	2	0	2	0	2	0	2	0	3	0
Hematopoiesis	0	0	0	0	0	0	0	0	0	0
Infarct	1	0	1	0	0	0	0	0	0	0
Abscess	0	0	0	0	0	0	0	0	0	0
Medullary hyperplasia	1	0	1	0	2	0	3	0	1	0
<u>Pituitary</u>	35	34	3	0	5	6	17	17	18	18
Hyperplasia	2	2	0	0	0	1	2	0	2	2
Cyst	1	0	0	0	0	0	0	0	0	0
<u>Thyroid</u>	32	34	2	2	0	0	16	12	19	19
Hyperplasia	1	0	0	0	0	0	0	0	0	0
Parafollicular hyperplasia	0	0	0	0	0	0	0	0	0	0
Thyroiditis	0	0	0	0	0	0	3	0	0	0

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(cont'd) page 2

	<u>Group 1</u>		<u>Group 2</u>		<u>Group 3</u>		<u>Group 4</u>		<u>Group 5</u>	
	UAREP	EPL	UAREP	EPL	UAREP	EPL	UAREP	EPL	UAREP	EPL
<u>Liver</u>	36	36	9	9	8	8	16	17	19	18
Bile duct proliferation	12	12	3	4	2	1	4	9	4	4
Lymphoid infiltrate	13	10	5	2	3	0	9	8	14	9
Hematopoiesis	1	0	0	0	0	0	0	0	0	0
Hepatocyte vacuolation	2	1	1	0	0	0	4	2	2	1
Necrosis/infarct	2	3	5	3	0	0	2	2	0	0
Fibrosis	6	7	0	0	0	1	3	0	2	0
Hepatitis	0	0	0	0	0	0	0	0	0	0
Abscess	0	0	1	0	0	0	0	0	0	0
Hyperplastic nodule/focus of change	0	0	0	0	0	0	0	0	0	0
<u>Kidney</u>	36	36	14	16	12	14	16	17	19	19
Interstitial nephritis	13	4	9	2	5	4	11	7	9	4
Glomerulosclerosis	22	3	6	0	8	0	11	1	9	4
Tubular dilatation	24	11	7	4	8	4	12	6	13	10
Tubular degeneration	0	0	0	1	0	0	0	1	0	4
Cortical cysts	3	0	6	0	1	0	2	0	0	0
Abscess	3	2	0	1	0	0	2	2	1	1

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(cont'd) page 3

	Group 1		Group 2		Group 3		Group 4		Group 5	
	UAREP	EPL	UAREP	EPL	UAREP	EPL	UAREP	EPL	UAREP	EPL
(Kidney cont'd)										
Pyelitis/ Pyelonephritis	1	1	1	0	2	0	4	2	6	0
Pelvic epithelial hyperplasia	2	4	0	0	1	0	2	0	2	6
Pelvic dilatation	1	0	1	1	1	0	0	0	0	0
<u>Lung</u>	36	35	22	21	18	18	17	17	19	19
Acute inflammation	33	0	17	0	16	0	15	1	19	3
Abscess	2	24	0	15	2	13	4	14	0	14
Chronic inflammation	34	18	20	4	15	6	15	3	17	7
Granulomatous inflammation	0	0	0	0	0	0	0	0	0	0
Congestion/ edema	13	6	2	1	8	1	4	2	6	0
Hemorrhage	0	0	0	0	2	0	0	0	1	0
Pneumocyte hyper- plasia/Alveolar macrophages	11	2	4	0	5	0	3	0	4	3
Fibrosis	0	0	0	0	0	0	0	0	0	0
<u>Heart</u>	32	36	2	2	0	0	17	17	19	19
Focal fibrosis	16	26	1	1	0	0	9	12	0	15
Mineralization	1	2	0	0	0	0	0	0	0	1
Focal myocarditis	3	1	1	0	0	0	1	1	5	1
Peri/Epicarditis	1	1	0	0	0	0	4	4	2	1
Endocarditis	0	1	0	0	0	0	0	0	2	0
Periarteritis	0	0	0	0	0	0	1	0	1	0

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(cont'd) page 4

	Group 1		Group 2		Group 3		Group 4		Group 5	
	UAREP	EPL	UAREP	EPL	UAREP	EPL	UAREP	EPL	UAREP	EPL
<u>Spleen</u>	35	36	4	3	0	0	17	17	16	17
Extramedullary hematopoiesis	0	3	1	0	0	0	1	5	1	9
Pigment	27	24	2	1	0	0	17	14	13	14
Reticuloendo- thelial cell hyperplasia	10	0	1	0	0	0	11	1	9	1
Lymphoid hyperplasia	3	1	0	0	0	0	4	0	1	0
<u>Skeletal Muscle</u>	36	36	2	0	0	0	0	0	13	19
Fibrosis	2	0	0	0	0	0	0	0	0	1
Myositis	1	0	0	0	0	0	0	0	0	0
<u>Peripheral Nerve</u>	28	36	0	0	0	0	1	0	19	18
Unremarkable	20	32	0	0	0	0	0	0	16	17
<u>Pancreas</u>	33	34	4	2	2	0	17	17	19	15
Fat necrosis	0	0	1	0	0	0	0	0	0	1
Fibrosis	0	1	0	0	1	0	0	1	4	6
Atrophy	0	0	0	0	0	0	0	0	0	1
Arteritis	0	0	1	2	1	0	0	0	1	0
Acute pancreatitis	0	0	1	1	0	0	1	1	3	2

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	<u>Group 1</u>		<u>Group 2</u>		<u>Group 3</u>		<u>Group 4</u>		<u>Group 5</u>	
	UAREP	EPL	UAREP	EPL	UAREP	EPL	UAREP	EPL	UAREP	EPL
<u>Bone</u>	5	1	2	0	0	0	0	0	6	0
Osteomalacia	0	0	0	0	0	0	0	0	0	0
<u>Bone Marrow</u>	11	11	2	1	0	0	0	0	8	9
Hematogenic activity	6	6	0	0	0	0	0	0	0	7
<u>Testis</u>	36	36	6	6	1	1	15	16	19	19
Level of activity	36	35	4	6	1	0	12	14	9	18
Vacuolation/ epididymis	2	2	1	1	0	0	0	2	0	1
Arteritis	2	1	2	0	0	0	3	3	0	0
Epididymitis/ Orchitis	3	0	1	0	0	0	1	0	0	0
Hemorrhage	0	0	0	0	0	0	0	0	0	0
Mineralization	1	2	1	0	0	0	0	0	1	0
<u>Seminal Vesicle</u>	34	35	1	1	1	1	17	17	15	16
Vesiculitis	5	1	0	0	0	0	2	1	0	4
Atrophy	1	2	0	1	0	0	0	4	0	3
<u>Prostate</u>	35	34	8	1	8	2	17	17	18	18
Atrophy	1	0	1	0	0	0	2	0	0	0
Prostatitis acute	5	4	1	0	3	2	5	4	6	2
Prostatitis chronic	4	3	0	0	0	1	1	0	2	0

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	<u>Group 1</u>		<u>Group 2</u>		<u>Group 3</u>		<u>Group 4</u>		<u>Group 5</u>	
	<u>UAREP</u>	<u>EPL</u>	<u>UAREP</u>	<u>EPL</u>	<u>UAREP</u>	<u>EPL</u>	<u>UAREP</u>	<u>EPL</u>	<u>UAREP</u>	<u>EPL</u>
<u>Urinary Bladder</u>	36	36	16	20	19	19	16	17	19	19
Cystitis	2	2	2	1	2	4	5	2	1	2
Calculi/mucus plug	5	3	3	0	3	0	1	1	5	0
Epithelial hyperplasia	0	0	0	0	0	1	1	0	0	0
Squamous meta- plasia	3	0	2	0	0	0	2	0	3	0
<u>Skin</u>	2	0	2	0	2	0	3	1	2	2
Cellulitis/ dermatitis	0	0	0	0	0	0	2	0	0	0
Ulceration	0	0	0	0	0	0	0	0	0	0
Epidermal in- clusion cyst	1	0	0	0	0	0	1	1	0	0

The numbers opposite each organ show total number of sections examined

APPENDIX IV-25D

COMPARISON OF SUMMARIES OF UAREP AND EPL HISTOPATHOLOGIC DIAGNOSES ON
NON-NEOPLASTIC CHANGES FOR FEMALE RATS TREATED WITH ASPARTAME WHICH
WERE FOUND DEAD OR SACRIFICED IN A MORIBUND CONDITION

(E-34, FIGURE 9A, PAGES 84-97)

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(cont'd) page 2

	<u>Group 1</u>		<u>Group 2</u>		<u>Group 3</u>		<u>Group 4</u>		<u>Group 5</u>	
	UAREP	EPL	UAREP	EPL	UAREP	EPL	UAREP	EPL	UAREP	EPL
<u>Liver</u>	33	34	7	6	6	5	26	26	29	30
Bile duct proliferation	10	16	3	1	0	0	2	4	1	7
Lymphoid infiltrate	14	14	5	3	1	0	13	13	13	7
Hematopoiesis	0	0	0	0	1	0	0	0	0	0
Hepatocyte vacuolation	3	2	0	0	2	2	1	2	3	2
Necrosis/infarct	4	3	1	2	1	0	3	6	2	2
Fibrosis	5	2	0	1	0	0	7	2	5	0
Hepatitis	2	3	0	0	0	0	0	0	0	3
Abscess	1	0	1	1	0	0	4	2	0	0
Hyperplastic nodule/focus of change	0	0	0	0	0	0	0	1	0	4
<u>Kidney</u>	34	34	13	13	11	11	25	26	27	30
Interstitial nephritis	3	3	2	3	3	1	2	3	3	1
Glomerulosclerosis	6	1	0	0	2	0	8	1	3	0
Tubular dilatation	9	3	4	3	3	1	6	2	8	2
Tubular degeneration	1	0	0	1	0	0	0	0	0	0
Cortical cysts	2	1	0	0	0	0	4	0	3	0
Abscess	2	1	1	1	1	1	2	1	1	0
Pyelitis/Pyelonephritis	7	2	1	0	2	0	2	1	0	0

Appendix IV-25D
(cont'd) page 3[illegible]

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(cont'd) page 6

(Lymph Node continued)	Group 1		Group 2		Group 3		Group 4		Group 5	
	UAREP	EPL	UAREP	EPL	UAREP	EPL	UAREP	EPL	UAREP	EPL
Reticuloendo- thelial cell proliferation	1	3	0	0	1	0	4	0	1	2
Lymphoid hyperplasia	2	0	0	0	1	0	9	0	5	1
<u>Salivary Gland</u>	34	0	0	0	1	0	25	25	24	29
Adenitis	0	0	0	0	0	0	0	0	0	0
<u>Brain</u>	33	33	17	1	20	3	26	4	28	29
Ventricle dilatation	1	2	2	0	2	0	5	1	2	1
Ventral compression	1	1	1	0	5	3	4	1	1	1
Glial prolif- eration	0	0	0	0	0	0	0	0	0	0
Encephalitis/ abscess	1	1	1	0	0	0	0	0	2	2
Meningitis	2	0	0	0	2	0	0	0	2	0
<u>Spinal Cord</u>	27	32	0	0	0	0	0	0	12	28
Unremarkable	18	15	0	0	0	0	0	0	10	21
<u>Mammary Gland</u>	28	32	9	9	8	7	22	26	26	26
Dilated ducts/ acini	12	12	1	1	0	0	5	8	8	13
Secretion	6	11	0	1	0	0	0	8	1	13
Mastitis	3	3	1	1	0	1	0	0	4	0
Periductal fibrosis	1	0	0	0	0	0	1	0	0	0

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	Group 1		Group 2		Group 3		Group 4		Group 5	
	UAREP	EPL	UAREP	EPL	UAREP	EPL	UAREP	EPL	UAREP	EPL
<u>Skin</u>	24	0	1	0	1	0	17	0	18	0
Cellulitis/ dermatitis	0	0	0	0	0	0	1	0	0	0
Ulceration	0	0	0	0	0	0	0	0	0	0
Epidermal in- clusion cyst	1	0	0	0	0	0	0	0	0	0

The numbers opposite each organ show total number of sections examined

APPENDIX IV-26
SIGNIFICANT DISCREPANCIES BETWEEN HISTOPATHOLOGIC DIAGNOSES

BY UAREP AND EPL ON E-33, 34

Group Set	Animal No.	Path No.	Organ	EPL Diagnosis	UAREP Diagnosis
1M	83-610	64-614	Testis	Interstitial cell tumor 3	0
1M	83-620	64-591	Pituitary	Adenoma	Hyperplasia 2
1M	83-621	64-592	Liver Adrenal Pituitary	Lymphoma 0 Adenoma	Focal lymphoid infiltrate 2 Pheochromocytoma Hyperplasia 3
1M	83-624	64-594	Pituitary Mammary gland	Adenoma Fibroadenoma	Adenocarcinoma Periductal fibrosis 2
1M	83-625	64-595	Pituitary	Adenoma	0
1M	83-628	64-623	Prostate	X	Chronic prostatitis 4
1M	83-631	64-598	Adrenal	Pheochromocytoma	Medullary hyperplasia 3
1M	83-635	64-599	Adrenal Liver	Nodular hyperplasia Hyperplastic nodule	Cortical adenoma Neoplastic nodule+
1M	83-637	64-630	Lung	0	Acute and chronic inflammation 3
1M	83-639	64-632	Bone marrow	0	Lymphosarcoma
1M	83-640	64-600	Thyroid	Parathyroid carcinoma	C-cell carcinoma
1M	83-641	64-633	Adrenal	X	Cortical adenoma
1M	83-646	64-601	Spleen	Lymphoma	Reticuloendothelial cell hyperplasia 3
1M	83-647	64-638	Subcutaneous tissue mass	0	Giant cell tumor
1M	83-649	64-639	Kidney	Abscess 5	0
1M	83-651	64-603	Brain	X	Astrocytoma
1M	83-652	64-604	Liver	0	Hyperplastic nodule (eosinophilic foci)
1M	83-653	64-605	Pituitary Spleen	Adenoma Extramedullary hematopoiesis 2	Hyperplasia 2 Reticuloendothelial cell hyperplasia 4
1M	83-656	64-606	Adrenal	Pheochromocytoma	Medullary hyperplasia 3
1M	83-662	64-609	Adrenal	0	Nodular hyperplasia 3
1F	83-668	64-809	Ovary	Oophoritis 4	0
1F	83-669	64-810	Ovary	0	Oophoritis 4
1F	83-671	64-836	Tissue mass	X	Subcutaneous sarcoma
1F	83-672	64-811	Liver	0	Neoplastic nodule
1F	83-673	64-837	Ovary	0	Granulosa cell tumor

X - indicates section was unremarkable
0 - indicates that no comparable diagnosis was recorded
1-5 degrees of severity of diagnosis as follows:
1-minimal
2-slight
3-moderate
4-moderately severe/high
5-severe/high

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Group Set	Animal No.	Path No.	Organ	EPL Diagnosis	UAREP Diagnosis *
1F	83-677	64-813	Adrenal Skin	0 Cellulitis 5	Cortical adenoma Heterotopic bone
1F	83-678	64-814	Pituitary Liver	0 Hyperplastic nodule	Hyperplasia 3 Neoplastic nodule *
1F	83-680	64-840	Lung Uterus	Alveolar adenoma 0	Lymphoma Endometritis 3
1F	83-684	64-818	Mammary gland	Adenocarcinoma	0
1F	83-685	64-819	Liver	Hyperplastic nodule	Neoplastic nodule *
1F	83-688	64-820	Adrenal	0	Pheochromocytoma
1F	83-690	64-822	Thyroid	Adenoma	C-cell carcinoma
1F	83-691	64-844	Spleen	Reticulum cell sarcoma	0
1F	83-693	64-845	Spleen	Reticulum cell sarcoma	0
1F	83-694	64-824	Ovary	Salpingitis 5	X
1F	83-695	64-846	Adrenal	Pheochromocytoma	Cortical hyperplasia 4
1F	83-696	64-825	Uterus	Fibrosis 3	Endometritis 3, squamous metaplasia 3
1F	83-697	64-847	Spleen	Reticuloendothelial cell hyperplasia 5	0
1F	83-698	64-848	Mammary gland	Adenocarcinoma	Fibroadenoma
1F	83-700	64-850	Heart Brain	0 0	Focal myocarditis 3, fibrosis 3 Meningitis 3
1F	83-701	64-851	Mammary gland	0	Fibroma
1F	83-703	64-853	Kidney	0	Pelvic epithelial hyperplasia 5
1F	83-709	64-826	Adrenal	0	Cortical adenoma
1F	83-710	64-859	Liver	Hepatitis 5	0
1F	83-713	64-827	Pituitary Liver	Adenocarcinoma Hyperplastic nodule	Adenoma Neoplastic nodule *
1F	83-714	64-862	Tissue mass (skin)	Hemangioma	0
1F	83-715	64-828	Pituitary	Hyperplasia 3	X
1F	83-716	64-863	Mammary gland Uterus	Fibroadenoma 0	0 Endometritis 3
1F	83-723	64-832	Thyroid Mammary gland	0 Adenocarcinoma	C-cell hyperplasia 3 Papillary adenoma
1F	83-725	64-867	Tissue mass (skin)	Hemangioma	0
2H	83-728	64-787	Lung Tissue mass	Metastatic tumor Fibrosarcoma	0 Fibroma
2H	83-729	64-788	Brain	0	Meningitis 3
2H	83-737	64-771	Pituitary	Hyperplasia 2	Adenoma
2H	83-742	64-797	Adrenal	Pheochromocytoma	Nodular hyperplasia 2
2H	83-745	64-775	Brain	0	Astrocytoma
2H	83-749	64-800	Liver	0	Lymphoma
2H	83-755	64-780	Skin	0	Cellulitis 3

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Group Set	Animal No.	Path No.	Organ	EPL Diagnosis	UAREP Diagnosis
2M	83-758	64-782	Skin	Epidermal inclusion cyst	Keratoacanthoma
2M	83-762	64-784	Pituitary	Adenoma	Hyperplasia 2
2F	83-766	65-011	Brain	0	Astrocytoma
2F	83-767	65-012	Pituitary	Adenoma	Adenocarcinoma
2F	83-769	64-989	Brain Mammary gland Uterus	0 0 0	Astrocytoma Fibroadenoma Endometritis 3
2F	83-771	64-990	Mammary gland	0	Adenoma
2F	83-773	64-991	Pituitary	Adenoma	X
2F	83-774	65-015	Lung	0	Abscess 4, acute inflammation 3
2F	83-775	65-016	Tissue mass	Fibrosarcoma	Neurofibroma
2F	83-778	65-019	Kidney	Tubular degen- eration 5	0
2F	83-779	64-992	Thyroid Liver	Adenoma Hyperplastic nodule	C-cell hyperplasia 5 Neoplastic nodule *
2F	83-780	64-993	Adrenal	Ganglioneuroma	Pheochromocytoma
2F	83-781	64-994	Uterus	Endometritis 3	X
2F	83-782	65-020	Tissue mass (skin)	Epidermal inclusion cyst	Keratoacanthoma
2F	83-784	64-996	Liver	0	Eosinophilic foci
2F	83-785	64-997	Adrenal	0	Medullary hyperplasia 3
2F	83-790	65-001	Liver	Hyperplastic nodule	Neoplastic nodule *
2F	83-792	65-003	Mammary gland	0	Fibroadenoma
3M	83-809	64-750	Brain	0	Meningitis 3
3M	83-811	64-751	Adrenal Pancreas	Pheochromocytoma 0	Medullary hyperplasia 4 Fibrosis 3, arteritis 3
3M	83-820	64-758	Adrenal	X	Cortical adenoma
3M	83-826	64-736	Spleen	Extramedullary hemato- poiesis 5	Reticuloendothelial cell hyperplasia 3
3M	83-837	64-764	Brain	0	Astrocytoma
3M	83-838	64-744	Lung	0	Chronic inflammation 3
3M	83-844	64-767	Pituitary	Hyperplasia 2	Adenoma
3F	83-852	64-971	Lymph node	Lymphosarcoma	Lymphoid hyperplasia 3
3F	83-855	64-952	Liver Mammary gland	0 0	Neoplastic nodule Fibroadenoma
3F	83-861	64-977	Liver Mammary gland Brain	0 0 0	Hepatocellular carcinoma Fibroadenoma Pituitary tumor involving base of brain
3F	83-866	64-980	Tissue mass (skin)	Squamous cell carcinoma	Keratoacanthoma
3F	83-867	64-981	Thyroid	0	Paraganglioma
3F	83-868	64-956	Tissue mass (skin)	Fibrosarcoma	Fibroma

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Group Set	Animal No.	Path No.	Organ	EPL Diagnosis	UAREP Diagnosis
3F	83-871	64-983	Mammary gland (tissue mass)	Secreting mammary gland	Fibroadenoma
3F	83-873	64-959	Pituitary	Adenoma	Hyperplasia 4
3F	83-874	64-960	Uterus (tissue mass)	Adenocarcinoma	Cystic endometrial hyperplasia 3
3F	83-875	64-961	Liver Ovary	0 Mesothelioma	Neoplastic nodule Granulosa cell tumor
3F	83-876	64-962	Tissue mass Mammary gland Ovary (tissue mass)	Lymphosarcoma Adenocarcinoma Squamous cell carcinoma	Lymphadenitis with cysts 4 Fibroadenoma Granulosa cell tumor
3F	83-881	64-963	Pituitary	Adenoma	Hyperplasia 2
3F	83-882	64-964	Mammary gland	0	Fibroadenoma
3F	83-883	64-965	Liver	Hyperplastic nodule	Neoplastic nodule *
4M	83-887	64-688	Liver	Hyperplastic nodule	Neoplastic nodule *
4M	83-888	64-712	Liver Brain	0 0	Neoplastic nodule Oligodendroglioma
4M	83-891	64-691	Pituitary	0	Adenocarcinoma
4M	83-892	64-713	Brain	0	Astrocytoma
4M	83-895	64-715	Brain Seminal vesicle	0 X	Astrocytoma Vesiculitis 4
4M	83-896	64-716	Adrenal	Pheochromocytoma	Medullary hyperplasia 2
4M	83-897	64-717	Liver	Bile duct proliferation 4	0
4M	83-901	64-695	Pituitary	Hyperplasia 3	Adenoma
4M	83-902	64-719	Adrenal	Pheochromocytoma	Medullary hyperplasia 1
4M	83-908	64-701	Pituitary	Adenoma	Hyperplasia 2
4M	83-912	64-704	Pituitary	Adenoma	Adenocarcinoma
4M	83-913	64-705	Liver Spleen	Bile duct carcinoma Extramedullary hematopoiesis 5	Neoplastic nodule Reticuloendothelial cell hyperplasia 2
4M	83-914	64-721	Brain	0	Abscess 3
4M	83-919	64-707	Brain	0	Astrocytoma
4F	83-926	64-922	Liver Spleen Mammary gland	0 Extramedullary hematopoiesis 5 Adenocarcinoma	Metastatic neoplasm Lymphoma Papillary adenoma
4F	83-927	64-908	Uterus	Fibrosis 3	Endometritis 3
4F	83-928	64-923	Mammary gland	Adenocarcinoma	Fibroadenoma
4F	83-934	64-926	Brain	Ependymoma	Astrocytoma
4F	83-936	64-928	Adrenal Ovary	Pheochromocytoma Oophoritis 4	Medullary hyperplasia 3 X
4F	83-940	64-931	Mammary gland	X	Fibroadenoma
4F	83-943	64-932	Adrenal	0	Cortical adenoma
4F	83-944	64-933	Uterus	Fibrosis 2	Endometritis 3
4F	83-947	64-915	Pituitary Liver	Adenoma 0	Adenocarcinoma Neoplastic nodule
4F	83-949	64-916	Liver	0	Eosinophilic foci
4F	83-952	64-918	Pituitary Heart	Adenoma Focal fibrosis 5	Adenocarcinoma Focal fibrosis 1

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Group Set	Animal No.	Path No.	Organ	EPL Diagnosis	UAREP Diagnosis
4F	83-962	64-944	Liver	Hyperplastic nodule	0
4F	83-963	64-945	Adrenal	Nodular hyperplasia	Cortical adenoma
4F	83-964	64-946	Kidney	0	Pelvic epithelial hyperplasia 3
4F	83-965	64-947	Liver	Parenchymal necrosis 5	Lymphoma
			Spleen	abscess 5 Extramedullary hemato- poiesis 5	Lymphoma
5M	83-966	64-648	Adrenal	0	Cortical adenoma
5M	83-972	64-650	Adrenal	Pheochromocytoma	Medullary hyperplasia 2
5M	83-976	64-675	Adrenal Spleen	Nodular hyperplasia Reticulum cell sarcoma	Cortical adenoma Reticuloendothelial cell hyper- plasia 3
5M	83-978	64-652	Pituitary	Adenoma	Hyperplasia 2
5M	83-979	64-676	Kidney Eye	0 0	Pyelitis 3 Acute keratitis with corneal ulceration 4
5M	83-987	64-680	Eye	0	Acute keratitis 4
5M	83-988	64-657	Thyroid	Adenoma	C-cell hyperplasia 3
5M	83-994	64-661	Adrenal	Pheochromocytoma	Medullary hyperplasia 4
5M	83-996	64-662	Brain	Meningioma	0
5M	83-999	64-665	Pancreas Stomach	0 X	Islet cell tumor Endocrine nodule
5M	84-001	64-684	Adrenal Kidney	Pheochromocytoma Pelvic epithelial hyper- plasia 4	Medullary hyperplasia 3 0
5M	84-002	64-667	Adrenal Pancreas	Pheochromocytoma X	Medullary hyperplasia 3 Islet cell tumor
5M	84-005	64-668	Pancreas	0	Islet cell tumor
5F	84-007	64-879	Brain	X	Pituitary tumor involving base of brain
5F	84-008	64-800	Mammary gland	0	Adenocarcinoma
5F	84-010	64-881	Brain	Meningioma	Medulloblastoma/meningeal sarcoma
5F	84-011	64-882	Spleen	Lymphosarcoma	Reticuloendothelial cell hyper- plasia 2
5F	84-012	64-883	Mammary gland	0	Fibroadenoma
5F	84-014	64-885	Brain Spleen	0 Reticuloendothelial cell hyperplasia 5	Focal encephalitis with abscess formation 0
5F	84-015	64-886	Adrenal Liver Brain	0 Hepatitis 5 Encephalitis	Cortical hyperplasia 5 Focal lymphoid infiltrate 1 X
5F	84-018	64-887	Adrenal	0	Nodular hyperplasia 3
5F	84-021	64-871	Uterus	Fibrosis 2	Benign tumor of vascular or smooth muscle origin
5F	84-023	64-891	Uterus	Endometritis 3	0
5F	84-025	64-892	Mammary gland	Adenocarcinoma	0
5F	84-026	64-893	Uterus	0	Endometrial hyperplasia 3

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Group Set	Animal No.	Path No.	Organ	EPL Diagnosis	UAREP Diagnosis
SF	84-031	64-898	Kidney	0	Abscess
SF	84-033	64-900	Brain	Meningioma	Meningoencephalitis
SF	84-034	64-901	Lymph node Brain	Necrosis 5 X	Lymphoid hyperplasia 3 Pituitary tumor involving base of brain
SF	84-035	64-902	Lymph node	0	Abscess 4
SF	04-036	64-873	Pituitary	0	Adenoma
SF	84-037	64-874	Mammary gland	Adenocarcinoma	Fibroadenoma
SF	84-038	64-903	Pituitary Lung Uterus	Adenoma 0 Endometrial polyp	Hyperplasia 4 Chronic inflammation 3 Endometritis 4
SF	84-039	64-904	Heart	Focal myocarditis 3	0
SF	84-041	64-875	Uterus	Fibrosis 4	Endometritis 3
SF	84-042	64-906	Liver Uterus	0 Fibrosis 4	Neoplastic nodule Endometritis 4
SF	84-043	64-876	Adrenal	Nodular hyperplasia	Cortical adenoma
SF	84-044	64-907	Mammary gland Liver	Adenocarcinoma Hyperplastic nodule	Duct papilloma Neoplastic nodule

*See text

APPENDIX IV-27

ORGANS EXAMINED MICROSCOPICALLY; A COMPARISON OF DATA FROM THE PROTOCOL,

HISTOPATHOLOGY INCIDENCE TABLE (HIT), EPL, AND UAREP

FOR CONTROL MALE AND FEMALE ANIMALS

Organ	Males				Females			
	Protocol	HIT	EPL	UAREP	Protocol	HIT	EPL	UAREP
Stomach	60	59	59	59	60	60	60	60
Small Intestine	60	59	57	56	60	60	59	59
Large Intestine	60	59	59	58	60	59	59	60
Lung	60	59	58	59	60	60	60	60
Heart	60	59	58	59	60	60	59	59
Kidney	60	59	59	59	60	60	60	60
Liver	60	59	59	59	60	60	60	60
Spleen	60	59	59	59	60	60	60	60
Pancreas	60	59	58	56	60	60	60	60
Pituitary	60	58	57	59	60	60	60	60
Thyroid	60	58	58	59	60	59	59	58
Adrenal	60	59	59	59	60	60	60	60
Gonad	60	59	59	59	60	59	59	60
Seminal Vesicle	60	57	58	58	--	--	--	--
Prostate	60	58	57	58	--	--	--	--
Uterus	--	--	--	--	60	59	57	60
Mammary Gland	10	11	11	10	60	60	58	56
Brain	60	59	58	58	60	60	59	60
Spinal Cord	60	59	58	59	60	57	58	58
Nerve	60	56	58	56	60	58	58	58
Muscle	60	58	59	59	60	59	57	60
Eye	60	59	59	59	60	60	59	59
Urinary Bladder	60	59	59	59	60	58	58	58
Salivary Gland	60	59	60	58	60	60	59	60
Lymph Node	60	59	55	56	60	59	56	57

HIT-Histopathology Incidence Table, a tabulation of sections prepared by HLA for microscopic examination.

APPENDIX IV-28

TYPES OF MINOR DISCREPANCIES BETWEEN HISTOPATHOLOGY INCIDENCE TABLES (HIT) AND
EPL MICROSCOPIC DIAGNOSES, GROUP 1 (CONTROL) WITH UAREP FINDINGS

Pathology No.	Animal No.	Organ	HIT	EPL	UAREP
64-589	83-613	Pituitary	x	---	P
64-594	83-624	Heart	x	---	P
64-600	83-640	(Total section discrepancy of 1; HIT)			
64-609	83-662	Lymph Node	x	N	P
64-611	83-606	(Total section discrepancy of 1; HIT)			
64-614	83-610	Thyroid	x	N	P
		Lung	x	---	P
64-615	83-611	Spinal Cord	x	N	P
64-616	83-612	Brain	x	---	P
64-618	83-615	Prostate	x	---	P
64-622	83-623	Small Intestine	x	N	P
64-625	83-630	Pancreas	x	---	P
64-629	83-636	Small Intestine	x	N	N
64-631	83-638	Lymph Node	x	N	N
64-632	83-639	Unusual Lesion	3	---	---
64-636	83-644	Femur (bone)	x	---	P
64-637	83-645	Pituitary	x	N	P
		Unusual Lesion	3	---	---
64-638	83-647	Lymph Node	x	---	P
		Unusual Lesion	x	---	P
64-646	83-663	Pancreas	x	N	N
64-815	83-679	Ovary	x	N	P
64-823	83-692	(Total section discrepancy of 1; HIT)			
64-831	83-721	Ovary	x	---	P
64-833	83-724	Ovary	x	---	P
		Uterus	x	N	P
		Salivary Gland	x	N	P
		Lymph Node	x	N	P
64-834	83-666	Salivary Gland	x	---	P
64-835	83-670	Salivary Gland	x	---	P
64-836	83-671	Small Intestine	x	N	P
		Salivary Gland	x	---	P
64-837	83-673	Salivary Gland	x	---	P
		Mammary Gland	x	N	---
		Bone Marrow	---	P	P
64-838	83-675	Heart	x	---	P
		Uterus	x	N	P
		Salivary Gland	x	---	P
64-839	83-676	Salivary Gland	x	---	P
64-840	83-680	Salivary Gland	x	---	P
64-841	83-683	Ovary	x	N	---
		Salivary Gland	x	---	P
64-842	83-686	Salivary Gland	x	---	P
64-843	83-687	Salivary Gland	x	---	P
		Lymph Node	x	N	N
64-844	83-691	Salivary Gland	x	---	P
64-845	83-693	Salivary Gland	x	---	P
64-846	83-695	Ovary	x	---	P
		Salivary Gland	x	---	P
64-847	83-697	Salivary Gland	x	---	P
		Nerve	x	N	P
		Muscle	x	N	P

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Pathology No.	Animal No.	Organ	HIT	EPL	UAREP
64-848	83-698	Salivary Gland	x	---	P
		Lymph Node	x	---	P
64-849	83-699	Uterus	x	---	P
		Salivary Gland	x	---	P
64-850	83-700	Salivary Gland	x	---	P
64-851	83-701	Salivary Gland	x	---	P
64-852	83-702	Salivary Gland	x	---	P
64-853	83-703	Salivary Gland	x	---	P
64-854	83-704	Salivary Gland	x	---	P
64-855	83-705	Salivary Gland	x	---	P
64-856	83-706	Salivary Gland	x	---	P
64-857	83-707	Salivary Gland	x	---	P
64-859	83-710	Salivary Gland	x	---	P
64-860	83-711	Salivary Gland	x	---	P
64-861	83-712	Salivary Gland	x	---	P
64-862	83-714	Brain	2	---	P
		Eye	x	---	P
		Salivary Gland	x	---	P
64-863	83-716	Salivary Gland	x	---	P
64-864	83-718	Salivary Gland	x	---	P
64-865	83-720	Salivary Gland	x	---	P
64-866	83-722	Salivary Gland	x	---	P
		Muscle	x	---	P
64-867	83-725	Salivary Gland	x	---	P

HIT = Histopathology Incidence Table, a tabulation of sections cut for microscopic examination

x = Section cut

P = Diagnosis indicating a section was present

N = No section

--- = No diagnosis listed

APPENDIX IV-29
INFREQUENT DISCREPANCIES NOTED BETWEEN CLINICAL OBSERVATIONS,
NECROPSY OBSERVATIONS, AND MICROSCOPIC EXAMINATION
GROUP 1 MALES AND FEMALES

Path Number	Animal Number	Discrepancy
64-629	83-636	Necropsy--thymus nodular, dark red Microscopic--no diagnosis listed for thymus
64-630	83-637	EPL diagnosis of lymphosarcoma for thymus. No gross lesions in thymus reported. Possible mislabeling of slide, and thymus is part of 64-629.
64-632	83-639	Necropsy--no tissue masses or "unusual lesions" described. HIT--3 sections of "unusual lesions" Microscopic--no appropriate diagnoses recorded for "unusual lesions"
64-638	83-647	Necropsy--subcutaneous tissue mass HIT--1 unusual lesion EPL microscopic--no corresponding diagnosis UAREP--subcutaneous mass; giant cell tumor
64-810	83-669	Necropsy--3 subcutaneous masses HIT--3 unusual lesions Microscopic--2 appropriate diagnoses, no corres- ponding diagnosis for "peri-anal lesion"
64-812	83-674	2 masses described as terminal clinical findings left and right axilla Necropsy--right axillary mass only described EPL micro-1 fibroadenoma
64-814	83-678	Necropsy--4 subcutaneous masses HIT--4 unusual lesions EPL microscopic--only 1 appropriate diagnosis. It is possible that all lesions are represented by one diagnosis.
64-819	83-685	These four represent similar discrepancies to that described above for 64-814.
64-829	83-717	
64-853	83-703	
64-829	83-717	

HIT=Histopathology Incidence Table of Sections Prepared

APPENDIX IV-30

INDIVIDUAL ANIMAL NECROPSY RECORD SHEET

The attached sheet shows the way in which information relative to autopsies was recorded at HLA. Tissues to be fixed and weighed were pre-marked. Tissues actually fixed, organ weights and gross finding are noted. Thyroid and adrenals were weighed after fixation. This was one of the males selected for section of mammary tissues. The initials of prosector and recorder are shown. The histology laboratory has recorded 29 tissues embedded in 12 blocks on this copy.

700-233 PROJECT NUMBER	Rat SPECIES	1 (Control) GROUP NO.	83-624 ANIMAL NO.
SAC <input checked="" type="checkbox"/> 12-14-71 DEATH <input type="checkbox"/> DATE 12-15-69 INITIATION DATE 104 WK. TERMINAL INTERVAL	ADMINISTRATION: <input type="checkbox"/> NO. DOSES <input checked="" type="checkbox"/> DIETARY <input type="checkbox"/> DERMAL <input type="checkbox"/> CAPSULE <input type="checkbox"/> INHALATION <input type="checkbox"/> INTUBATION <input type="checkbox"/> OTHER <input type="checkbox"/> INTRAVENOUS METHOD OF SACRIFICE: Diabulal Exsanguination		Male SEX 64-594 PATH NO.
12,237B L. H. NO.	SC 18862 COMPOUND	0 LEVEL	Sm. An. Tox. LABORATORY

TISSUES TAKEN	REMARKS:	TERM WT. 543
<input checked="" type="checkbox"/> BRAIN	Tissue Mass - left axilla subcutaneous nodular, pink- ish-white, section in jar. Pituitary - enlarged, grey with dark red areas.	ORGAN WEIGHTS
<input checked="" type="checkbox"/> PITUITARY		
<input checked="" type="checkbox"/> SPINAL CORD		
<input checked="" type="checkbox"/> EYE R		BRAIN
<input checked="" type="checkbox"/> SAL GLAND Submax.		
<input checked="" type="checkbox"/> THYROID		THYROID 0.045
<input type="checkbox"/> PARATHYROID		
<input type="checkbox"/> OESOPHAGUS		LUNG
<input checked="" type="checkbox"/> LUNG	Lungs - distended with abcesses	HEART 2.15
<input checked="" type="checkbox"/> HEART		
<input type="checkbox"/> AORTA		
<input checked="" type="checkbox"/> LIVER	areolae	LIVER 14.00
<input type="checkbox"/> GALLBLADDER		
<input checked="" type="checkbox"/> SPLEEN	Right Adrenal - greatly enlarged	GALLBLADDER
<input checked="" type="checkbox"/> KIDNEY	nodular, pale pink with	SPLEEN
<input checked="" type="checkbox"/> ADRENAL	dark red areas, in jar	KIDNEY 3.60
<input checked="" type="checkbox"/> STOMACH		* R-1.61
<input checked="" type="checkbox"/> PANCREAS		ADRENAL 2.0.51
<input checked="" type="checkbox"/> S. INTESTINE	Kidneys - medulla dark red	
<input type="checkbox"/> DUODENUM		PANCREAS
<input type="checkbox"/> JEJUNUM		
<input type="checkbox"/> ILEUM		
<input checked="" type="checkbox"/> L. INTESTINE	+ mucus	CECUM
<input checked="" type="checkbox"/> LYMPH NODE Mes.	29 (12) RIL	U. BLADDER
<input checked="" type="checkbox"/> U. BLADDER		
<input checked="" type="checkbox"/> TESTIS c Eppid.	1-13-72	TESTIS c Eppid. 4.36
<input checked="" type="checkbox"/> PROSTATE		
<input checked="" type="checkbox"/> SEM. VESICLE		OVARY
<input type="checkbox"/> OVARY		
<input checked="" type="checkbox"/> UTERUS p. 5.6		TISSUE MASS 4.11
<input checked="" type="checkbox"/> SEM. VES. TISS.		
<input checked="" type="checkbox"/> BONE MARROW		x Sem. Ves. 2.74
<input checked="" type="checkbox"/> BONE MARROW Sternum		x Prostate 1.80
<input checked="" type="checkbox"/> NERVE MUSCLE		
<input checked="" type="checkbox"/> TISSUE MASS		

SINGLE ANIMAL AUTOPSY SHEET HL NO. 382 Rev. 6/65

*After fixation

TECHNICIAN

E.T. / K.9

0.7202

PART 1 - PROTOCOL COPY
PART 3 - BICMETRICS COPY

PART 2 - COORDINATOR COPY
PART 4 - HISTOLOGY-PATHOLOGY COPY

CHAPTER V

E-70: LIFETIME TOXICITY STUDY OF ASPARTAME IN THE RAT

INTRODUCTION

Searle Laboratories contracted with Hazleton Laboratories to conduct this study (PT 892H72; FDA Entry Book No. E-70) for the purpose of evaluating and characterizing the effects of feeding aspartame (SC-18862) to albino rats throughout gestation and lactation, with treatment continuing for 104 weeks after weaning. This report covers only the 104 week post-weaning treatment phase (Hazleton Project No. 700-240), whereas the parents of these rats were fed aspartame for 60 days at the same dose level prior to mating, and throughout their gestation. These rats are part of the first generation offspring of the two generation reproductive study reported to FDA as E-11 (Chapter XI of this report). Aspartame was fed at two dosage levels to Charles River cesarean-derived (CRcd) strain of rats for 104 weeks after weaning, during which time clinical observations, body weight changes, food and compound consumption, ophthalmoscopic observations, and various hematology, clinical and special chemistries, urinalysis, histopathologic, necropsy and other observations were made on the animals. The oral toxicity trial began January 6, 1971 and terminated with sacrifice of the rats on January 3-12, 1973.

Personnel

The Searle preclinical safety study protocol design committee included the following individuals:

Dr. Dutt.....Biostatistician
Dr. F. Saunders.....Bioresearch Director
Dr. Ranney.....Drug Metabolism Representative
Dr. Polk.....Clinical Representative
Dr. Rao.....Path Tox Department Monitor
Dr. McConnell.....Path Tox Department Advisor

Dr. D. W. Jessup was Project Coordinator and Dr. Frederick E. Reno was Project Manager for Hazleton Laboratories. The histopathology diagnoses were made by Drs. James F. Ferrell and William B. Busey of Experimental Pathology Laboratories, Herndon, Virginia.

Experimental Animals and Conditions

Rats were divided into three groups--control, low dosage (2 g/kg body weight/day) and high (4 g/kg body weight/day). There were 60 females and 60 males in the control groups, with 40 males and 40 females in each of the two treatment groups.

Animals for this study were obtained from the litters of the two generation reproductive study of which eight and one-half pups per litter were available for assignment to this project. Since 20 litters per group were available (170 pups), 80 weanlings (40 male and 40 female), were selected (four per litter) per treatment group and 120 weanlings (60 male, 60 female) were selected (six per litter) for the control groups. Thus, the protocol design precluded random distribution of pups based on genetic constitution, although the parents of the weanlings were from a random-outbred colony.

The rats were housed individually in wire mesh cages. They had free access to chlorinated water and to Purina Laboratory Chow with which the appropriate amounts of aspartame were mixed. Other matters relating to animal feeding and care are described in Chapter II.

General Comments on Protocol and Amendments

Copies of the protocols and amendments are contained in Appendix V-1. The earliest protocol relative to E-70 is contained in Hazleton Laboratory Project Sheet No. 1 dated July 15, 1970 (Appendix V-1, Item A). The earliest Searle protocol was transmitted December 28, 1972 with the statement that "The original protocol (attached; Hazleton Labs format; 7-15-70) has been reduced to the current format employed by Searle Labs, and a copy is attached. Addition of amendment one thus provides a complete current protocol."

This Searle protocol added five additional clinical chemistries to be performed terminally, one chemical analysis to be done on frozen liver tissue at the termination of the experiment and a revision of the necropsy and histopathology protocol. Hazleton protocol sheet No. 2 dated January 10, 1973 (Appendix V-1, Item C) provided the mechanism to carry out some, but not all, of the additional determinations called for in Searle amendment number 1. A handwritten Hazleton internal memorandum dated February 2, 1973 provided for carrying out the additional procedures requested in the Searle amendment of December 28, 1972. It is of passing interest that the experiments were to terminate beginning January 3, 1973, but the Hazleton memoranda implementing the collection of specimens at the end of the experiment, were dated January 10 and February 2, 1973.

Statistical Evaluation of Data: The Hazleton Project Sheet No. 1 dated July 16, 1970 was less specific than the Searle protocol dated December 28, 1972. The latter stated on page 4 that body weight change; food and drug consumption; and all clinical laboratory values should have group means \pm standard error with appropriate analysis of intergroup variance at each time interval. For the mean incidence and onset of neoplasms, there should be appropriate analysis of intergroup variance at termination using the life table method. Problems of statistical analysis are discussed in more detail in Chapters II and IV.

Interim and Final Reports: In addition to the final report, the Searle protocol specified that the sponsor (Director: Path-Tox Department) requires a brief quarterly report relating to the statistically significant changes in terms of hematology, urinalysis, clinical chemistry, with a general statement on observations, physical examinations, and postmortem observations. This report was to be received on the first of January, April, July, and October. When UAREP requested copies of such reports from Searle and Hazleton, the only reply received was from Hazleton to the effect that the reports had been submitted to Searle.

RESULTS AND DISCUSSION

Clinical Observations

Examinations for gross signs of toxicity, pharmacological effects, and the incidence, size, and location of tumors, were recorded at the same interval as body weight and food consumption. Rats were observed daily for mortality and morbidity. General physical external observations included digital palpation for protruding masses and examination of body orifices and extremities. No further neurological observations were specified.

A summary of palpable nodules, tissue masses, or skin lesions is given in the table presented on page 37 of Entry Book E-70. UAREP noted a few minor discrepancies in this table, compared with clinical observations as recorded on individual animals (Appendix V-2). UAREP checked the reports of clinical observations for variations in reporting, a listing of which is given in Appendix V-2. As discussed in Chapter II, UAREP does not necessarily equate these variations as problems in observation and recording, inasmuch as it is not possible at this time to differentiate such matters from the normally occurring physiologic variations which at times appear and disappear.

Body Weight Gains

Individual body weights were determined weekly for the first 26 weeks, bimonthly for the succeeding 26 weeks, and once every four weeks thereafter. The INTEC printout for body weights agreed with this schedule. Entry Book E-70, Appendix Table No. 1, Appendix pages 3,4 summarized body weights at 0, 1, 2, 4, and every 4 weeks for week 8 through 104.

A summary of UAREP computed body weight means, and standard deviations and selected values from E-70, are given in Table 5-1. Of the 24 means and 24 standard deviations, there were 33 complete agreements, seven with a deviation of one number or within 1% and only one varied by more than 5%.

The increases in body weight were significantly ($P < 0.05$) reduced for the high dose male groups at the 26 and 52 week intervals as shown in Appendix V-3, but not at the 104 week interval. UAREP also found that there was a significant depression in the Group 3 male body weights as compared with the Group 2 weights at the 5% probability level. This agrees with the results reported in Entry Book E-70.

Food Consumption

Food consumption, based on the weight changes in food containers, was recorded at the same intervals as body weights. UAREP sampled the food consumption data for the first 24 weeks. Of the 47 comparisons for which data were available, 44 of the UAREP determinations were within 2% of the Hazleton report and 31 of the 44 were identical. Because of the extensive analysis of food consumption carried out on earlier and later experiments, as reported in Chapter IV and Chapter VII, a more extensive analysis was not done by UAREP for E-70. Minor variations such as noted are not considered critical in terms of the overall results of the experiment.

Compound Consumption

Aspartame was mixed in the diet which was freely available to the rats. The low dose of 2 g/kg/day was considered to be 67 times and the high dose of 4 g/kg/day 133 times the expected human dose. Aspartame

Table 5-1

Comparison of Mean Body Weight \pm Standard Deviation in Grams for Rats at
Weeks 0, 28, 52, and 104 as Reported in E-70 Table No. 1,

Appendix pages 3-4, and as Computed by UAREP

Interval (week)	Male Groups			Female Groups		
	<u>1</u>	<u>2</u>	<u>3</u>	<u>1</u>	<u>2</u>	<u>3</u>
0-HLA	84 \pm 21	83 \pm 20	82 \pm 11	76 \pm 22	79 \pm 13	76 \pm 12
0-UAREP	84 \pm 21	83 \pm 20	82 \pm 11	76 \pm 22	79 \pm 13	76 \pm 12
28-HLA	560 \pm 63	567 \pm 49	528 \pm 62	323 \pm 36	328 \pm 29	313 \pm 46
28-UAREP	559 \pm 63	567 \pm 49	528 \pm 62	330 \pm 59	328 \pm 29	313 \pm 46
52-HLA	585 \pm 75	588 \pm 53	547 \pm 72	354 \pm 43	351 \pm 35	336 \pm 51
52-UAREP	585 \pm 75	588 \pm 53	545 \pm 71	354 \pm 43	351 \pm 35	336 \pm 51
104 HLA	527 \pm 102	541 \pm 90	521 \pm 98	402 \pm 101	391 \pm 49	388 \pm 80
104-UAREP	520 \pm 100	541 \pm 90	515 \pm 100	394 \pm 102	389 \pm 47	388 \pm 81

was mixed into the basal diet of laboratory chow each week.

For purposes of calculating the addition rate necessary to maintain the specified dietary intake level, the 17 lots of aspartame Searle provided to Hazleton Laboratories for this trial were considered to be 100% pure. The content of diketopiperazine (SC-19192) varied from 0.40% to 1.50% (Entry Book E-70, Table 1A, Appendix p 5). Such small amounts of DKP would be inconsequential in terms of any overall effect upon the experiment. Because UAREP checked the compound consumption figures in detail in earlier and later experiments, such computations were not checked in E-70 because the minor variations that might be observed would not be important in their effect on the overall experiment.

Survival

As was the situation in E-33,34, in these experiments the Searle protocol and the Hazleton Project Sheets gave little specific information as to the manner in which survival data would be collected, summarized, or presented in the final report. Entry Book E-70, page 15 stated that a life table technique for survival was to be used in statistical evaluation. Table 5-2 compares the mean survival rates and survival times as determined by Hazleton and UAREP at 104 weeks. The data presented are similar, but differ in some instances. The mean survival time as computed by UAREP agreed completely with Hazleton for Group 1 and Group 2 males, with degrees of variation noted for the other groups. Although the percent survival data on page 22 show 104

Table 5-2

Comparison of UAREP and HLA Data (E-70, page 22) for Mean Percent Survival \pm Standard Error, Mean Survival Time in Days and Percent Survival at Selected Intervals

A.. % Survival Rate at 104 Weeks

Group	Males		Females	
	HLA % S.E.	UAREP % S.E.	HLA % S.E.	UAREP % S.E.
1	41.7 \pm 6.4	36.6 \pm 7.1	48.4 \pm 6.5	43.1 \pm 7.3
2	50.0 \pm 8.0	47.7 \pm 8.5	45.0 \pm 7.9	40.4 \pm 9.0
3	57.5 \pm 7.9	55.2 \pm 8.5	52.5 \pm 7.9	45.3 \pm 9.2

B. Mean Survival Time (Days)

Group	Males		Females	
	HLA	UAREP	HLA	UAREP
1	643	643	649	660
2	654	654	632	641
3	650	662	661	659

Analysis of Variance and Life Table Analyses showed no significant differences in survival between any groups.

Table 5-2
continued

C. Percent Survival at Selected Intervals

Males

<u>Interval</u>	¹		²		³	
	<u>HLA</u>	<u>UAREP</u>	<u>HLA</u>	<u>UAREP</u>	<u>HLA</u>	<u>UAREP</u>
13	100	100	100	100	100	100
26	100	100	100	100	100	100
52	100	100	100	100	100	100
78	82	83	85	85	80	80
91	57	57	70	70	68	68
104	42	37	50	48	58	55

Females

<u>Interval</u>	¹		²		³	
	<u>HLA</u>	<u>UAREP</u>	<u>HLA</u>	<u>UAREP</u>	<u>HLA</u>	<u>UAREP</u>
13	98	98	100	100	100	100
26	98	98	98	100	98	98
52	95	95	90	92	95	95
78	87	87	80	82	85	85
91	78	78	73	74	78	78
104	48	43	45	40	53	45

weeks, the copies of computer tapes made available to UAREP appeared to show the analysis only going to 103 weeks. The terminal figures for percent survival differed significantly for the UAREP and HLA life table analyses as they went beyond 100 weeks (Table 5-2C). Although UAREP percentages were less, they consistently shifted in the same direction. The reasons for this were not apparent to UAREP nor was the reason that the computer tapes only went to 103 weeks. Although the methods employed apparently differed somewhat, both the Entry Book and UAREP agree that there were no statistically significant differences in survival between male or female rats fed aspartame at either dosage level or their respective controls.

Clinical Laboratory Studies

Five male and five female rats from the control and each treatment group were evaluated at 6, 13, 26, 52, and 104 week intervals for hematologic and clinical chemistry parameters.

A list of animals used for collection of blood samples for hematology and clinical chemistry determinations is contained in Appendix V-4A and B. Normally, the first five animals in numerical order from each of the groups were used for collecting blood at each interval. When an animal was removed from the experiment (death, accidental death, escape, etc.) the next animal in numerical order was added to the group to maintain a total of five animals being sampled. The only exception to the above procedure for hematology specimens noted in E-70 was in Group 1 females, where the first two animals were skipped during the initial

interval, with the first animal (90968) in numerical order being sampled at all subsequent intervals. The second animal in numerical order (90869) died during the second week interval and hence was not sampled at week 6. The first animal was indicated as normal and no reason was given in the material provided UAREP for not including this animal in the initial group of animals sampled for hematology. Of the initial five males sampled per group, only 2, 2, and 4 were alive at the terminal interval for Groups 1, 2, and 3 respectively. Only 1, 4, and 3 of the females initially sampled were sampled at the termination of the experiment in Groups 1, 2, and 3, respectively.

Blood for clinical chemistries was collected by Unopettes from the tail vein using a segmental amputation procedure at the 6, 13, 26, and 52 week intervals, and by aorta puncture at the 104 week terminal interval. Rats to be used as blood sources for clinical chemistries were apparently selected as the second series of five animals based on the numerical sequence following rats used for hematology determinations. This seemed to be consistently carried out in this manner with the following exceptions: (1) Group 1 female animal number 90874 was sampled for hematology at week 6 and then sampled for clinical chemistry determinations subsequently until it died, and (2) Group 1 female animal number 90876 was sampled for clinical chemistry determinations at weeks 6, 13, and 52 and missed at week 26.

Table 5-3 summarizes the animals that were used for the blood sources for hematology and clinical chemistry determinations at various different intervals. The procedure for determining which animals were to be sampled for the different types of determination was complicated

Table 5-3

Sampling Patterns of Rats Used for Hematology and
Clinical Chemistries

<u>Group</u>	<u>Animal number</u>	<u>Weeks at which Rats were Sampled</u>	
		<u>Clinical Chemistries</u>	<u>Hematology</u>
1M	90813	6, 13, 26, & 52	104
1M	90814	6, 13, 26, & 52	104
1M	90816	6, 13, 26, & 52	104
2M	90933	6, 13, 26, & 52	104
2M	90934	6, 13, 26, & 52	104
2M	90935	6, 13, 26, & 52	104
3M	91013	6, 13, 26, & 52	104
1F	90874	13, 26, & 52	6
1F	90876	6, 13, (26) ¹ , 52	
1F	90875	6, 13, 26, & 52	104
2F	90973	6 & 13	26, 52, & 104
3F	91053	6, 13, 26, & 52	104
3F	91054	6, 13, 26, & 52	104

¹ Rat 90876 was not sampled for either hematology or clinical chemistry at 26 week interval.

by the fact that animals were removed from the experiment for various reasons (death, accidental deaths, escape, etc.), and by shifting animals used for clinical chemistry determinations to hematology determinations. The instances in which the same animal was sampled at all five intervals is especially low for the clinical chemistries. Of the six groups of five rats (30 total) sampled at the first interval, only three of the same 30 rats were sampled at the terminal interval because they had either died or were transferred to the hematology series. Fifty-nine different rats were used in obtaining the series of 30 samples for the clinical chemistry series.

Hematology - The following hematology parameters were specified in the Hazleton Project Sheet dated July 15, 1970, to be determined at the 6, 13, 36, 52, and 104 week intervals: hematocrit, hemoglobin, erythrocyte count, leukocyte count, and differential leukocyte count. Appendix Table No. 2 of Entry Book E-70 summarizes Hazleton's results for these hematology parameters.

Confidence intervals based on control group mean values observed in the respective treatment groups are summarized in Appendix V-5. Of the 20 means in each of Groups 2 and 3 for hematocrit, hemoglobin, red cell and white cell counts, there were one, three, four, and eight means, respectively, which fell outside the computed confidence intervals ($P < 0.05$). UAREP does not wish to overemphasize the significance of the confidence interval. However, it is another statistical tool which gives an indication of the variability of the data due to a variety of factors including normal biologic variation, experimental conditions,

specimen collection, handling and analysis, and experiment design. One could surmise from Appendix V-5 that more of the white blood cell count data fell outside the range in which one would expect to find 95% of normal values than it did for hematocrit readings.

A summary of the discrepancies between UAREP's validation study and Appendix Table No. 2 of Entry Book E-70 is contained in Appendix V-6. No transcriptional errors were noted when UAREP compared the 600 pieces of hematology data recorded in Appendix Table No. 2 of Entry Book E-70 to that of the hematology laboratory notebooks, which were their earliest data source.

The following discrepancies were noted in the 240 means and standard deviations computed and recorded in Appendix Table No. 2 of Entry Book E-70. Eight inconsequential rounding discrepancies were found by UAREP. No computational discrepancies were noted between UAREP's validation of data and the Entry Book.

A comparison of the results of the [•]t-test in Appendix V-7 shows that in 13 instances, both HLA and UAREP had significant differences in means. In three instances, HLA reported significant differences and UAREP did not, although the values were close to the $P < 0.05$ level. In one instance, UAREP's result was significant and HLA's was not. UAREP found four significant differences by t-test in which HLA did not attempt to compare the same groups. In 10 instances in which HLA reported a significant t-test, UAREP had positive ANOVA, LSD, and Q tests all at $P < 0.05$; in five instances none were significant.

Clinical and Special Chemistries - The initial experimental design as contained in Hazleton Project Sheet No. 1 called for the following

clinical chemistry determinations to be performed at the 6, 13, 26, 52, and 104 week intervals; fasting blood glucose, blood urea nitrogen, total serum protein, total bilirubin, serum glutamic pyruvic transaminase, serum alkaline phosphatase, and electrophoresis of serum proteins. In addition to the above parameters, the following were to be determined at the 104 week (terminal) interval: serum sodium, calcium, potassium, carbon dioxide, chloride, and serum glutamic oxalacetic transaminase (SGOT). An amendment at the end of the experiment added serum insulin, total cholesterol, triglycerides, and liver phenylalanine hydroxylase.

Serum Glucose, BUN, SGPT, Alkaline Phosphatase, and Total Bilirubin: In UAREP's validation of these determinations in Appendix Table 3 of Entry Book 70, pages 20-29, there were no transcriptional discrepancies detected when comparing the 900 bits of data with the earliest data source available to UAREP. There were no computational discrepancies in the 180 means and 180 standard deviations, although four of the standard deviations showed inconsequential rounding discrepancies (Appendix V-8).

UAREP found significant t-tests on seven of the eight reported by HLA. Two of these had ANOVA results greater than $P > 0.05$. Based on UAREP's statistical analysis of clinical chemistry data using the Analysis of Variance, five additional interactions between treatment groups which HLA did not compare, were found to be significant at the 5% level of probability. These are summarized in Appendix V-9.

The confidence intervals computed by UAREP based on the control variance together with the means of the various treatment groups, are

presented in Appendix V-10. These intervals show a wide range because of the large standard error of the small control groups.

Serum Sodium, Potassium, Chloride, Calcium, and Carbon Dioxide: Entry Book E-70, Appendix Table No. 3, pages 30-31 summarizes the results of the serum determinations of sodium, potassium, chloride, calcium, and carbon dioxide. Appendix V-11 presents the computed means of treatment groups along with the confidence interval ($P < 0.05$) based on the control values. The sodium values for Group 1 males seem low when compared with normal values and the values observed in the various treatment groups. The potassium was observed to be low in all treatment groups. Serum chloride and calcium values were within the normal expected limits.

In the 150 recorded observations, UAREP found no transcriptional discrepancies between the earliest data source and the figures contained in the Entry Book. Of the 30 computed means and 30 standard deviations in the Entry Book, UAREP found only one computational error in a standard deviation and four inconsequential rounding errors of which none would affect the interpretation of results (Appendix V-12).

Electrophoresis: Electrophoresis of the protein components of serum from each of the experimental groups were determined at the 6, 13, 26, 52, and 104 week intervals. A summary of means and confidence intervals computed at the 95% probability level is presented in Appendix V-13. Some of the intervals show a remarkably large range based on the variability of the control values. Up to half of the means in some groups fall outside the confidence interval. UAREP's validation of the electro-

phoresis data determined that a number of discrepancies were present in Appendix Table No. 3 (pp 35-44) which are summarized in Appendix V-14. Nine hundred and thirty electrophoresis observations were recorded in which UAREP reviewed and noted no transcriptional errors in transferring the data from the laboratory data sheets to the Entry Book Appendix Table No. 3. The summary of electrophoresis results in the Entry Book, E-70, pages 35-44 involved computing 186 means and 186 standard deviations. In all these, UAREP noted only seven inconsequential rounding discrepancies, which had no effect on interpretation of results.

Entry Book reported 12 statistical interactions ($P < 0.05$) between groups. UAREP t-test confirmed these. UAREP found that seven of the 12 t-tests reported in the Entry Book not to be significant at the 5% level of probability based on Analysis of Variance. UAREP noted two additional interactions between treatment groups when the data were evaluated statistically and these interactions are presented in Appendix V-15.

Triglycerides, Cholesterol, Insulin, and Liver Phenylalanine Hydroxylase: The computed means along with the confidence intervals for the triglycerides, cholesterol, and insulin are given in Appendix V-16.

UAREP found no transcriptional errors in the 60 values recorded for cholesterol and triglycerides as determined at the 104 week interval and reported in Appendix Table No. 3, pages 32-33 and Figure No. 3, p 32. Two inconsequential rounding discrepancies were noted in the 12 means and 12 standard deviations presented and these are listed in Appendix V-17.

Of the 30 insulin values reported, three inconsequential rounding discrepancies were detected when UAREP compared the earliest data source

for the insulin determinations against Entry Book E-70. The only statistically significant interaction reported in the Entry Book was confirmed by a UAREP t-test but not by the Analysis of Variance at the 5% level of probability. One computational error was noted which also caused two additional computational errors to be made in the summary of the data (Appendix V-17).

UAREP's review of the raw data on liver phenylalanine hydroxylase activity, Appendix V-1, Item A, agreed completely with the data reported in E-70, Figure 7A, page 73. These results are summarized on E-70, page 36, but the significance is not discussed of "the compound-related increase in enzyme activity for the low level males and for the high level males and females." UAREP confirmed the statistical significance of this increase in liver phenylalanine hydroxylase (Appendix V-18).

Urinalysis - All of the tests performed on urine, except for specific gravity, utilized Ames dipsticks which gave qualitative results not suitable for statistical analysis. Although the measurements of specific gravity are quantitative, a meaningful statistical evaluation can only be made if the urine of high specific gravity is diluted so that a better numerical result is available. At 104 weeks, the specific gravity readings of the male Group 2 appear lower than either Group 1 or 3. A more meaningful analysis could not be carried out since there were no dilutions of the higher concentration. UAREP's review of the urinalysis data presented in Entry Book E-70, Appendix Table 4, did not reveal any discrepancies.

Ophthalmoscopic Examination

Ophthalmoscopic examinations were scheduled for all animals prior to initiation, at one year, and at the termination of the experiments. UAREP validation of ophthalmoscopic examinations revealed no apparent discrepancies between the summary reports submitted by individuals performing the examinations and the results reported in Appendix Table No. 5, pages 58-60 of Entry Book E-70. The only apparent lack of consistency was that the protocol specified that eye examinations were to be performed prior to the initiation of the experiment and they were actually performed two weeks after the experiment began. This, however, would not alter the results of the study.

Necropsy; Organ and Organ/Body Weight Ratios; Gross Diagnoses

The initial procedures to be carried out at autopsy were delineated in the Hazleton protocol (Appendix V-1-Item A) and were subsequently modified as shown in Appendix V-1-Item B. The principal changes included the following:

- 1) The initial plan to prepare microscopic sections on only five males and five females from each group was changed to provide histopathologic examination of tissues from all animals.
- 2) Weighing of spleen, urinary bladder, and tissue masses was deleted.
- 3) The weighing of adrenal gland was added.

The protocol listed 30 organs to be fixed, of which 10 were weighed at necropsy (Appendix V-1).

Organ and organ/body weight ratios are summarized in E-70, Appendix Table 7, pages 68-72. These were carefully checked by UAREP and the discrepancies noted are summarized in Appendix V-19. Of the 96 means and 96 standard deviations recorded in Appendix No. Table 7, UAREP noted only five inconsequential rounding, and one minor computational, discrepancy as summarized in Appendix V-19. UAREP and HLA agreed in all instances on the significance of the 64 group comparisons of means.

The necropsies were performed by laboratory personnel. Terminal sacrifice of surviving animals was done on the dates indicated in Table 5-4 which began on the day two years after the experiment began and continued for nine days. The fact that equal numbers of rats from each group were not sacrificed each day would not produce significant problems. It was presumed that all rats were assumed to undergo terminal sacrifice on the same date for purposes of computing survival time of various groups.

Histopathologic Findings

The objectives and methods relating to the histopathology review of slides for E-70 are similar to those described for E-33,34 in Chapter IV under the same chapter subtitles.

Comparison of EPL and UAREP Diagnoses

The methods of tabulating and comparing histopathologic diagnoses in E-70 are identical to those described in Chapter IV and the same UAREP pathologists were responsible for reviewing the slides.

Table 5-4

Dates on Which Varying Numbers of Surviving Male (M) and
Female (F) Rats Were Sacrificed

<u>Date</u>	Group 1		Group 2		Group 3		<u>Total</u>
	<u>M</u>	<u>F</u>	<u>M</u>	<u>F</u>	<u>M</u>	<u>F</u>	
1-03-73	2	0	1	0	0	0	3
1-05-73	3	5	4	5	5	5	27
1-09-73	10	10	5	5	0	0	30
1-10-73	4	5	5	6	5	5	30
1-11-73	3	9	5	0	8	0	25
1-12-73	0	0	0	0	4	10	14
Total Sacrificed 1/3-12/73	22	29	20	16	22	20	
Death after 1/2/73	3	0	0	2	1	1	

For E-70, histologic slides were prepared at Hazleton Laboratories and then transferred by subcontract to EPL for histopathologic diagnosis by two pathologists, John F. Ferrell, DVM, and William M. Busey, DVM, Ph.D.

Tumors - A tabulation of tumor frequencies is shown in Appendix V-20, which gives the total numbers of tumors according to type and site of occurrence and also shows a comparison between UAREP and EPL by experimental groups. As before, the total numbers of tumors shown in this table and that shown by EPL in the original report, Figure 5, pages 38-41 may differ, as UAREP employed different methods of tabulating. All lymphoreticular and hematopoietic tumors are grouped under the single heading of "lymphomas" and no specific site or origin is denoted. An animal showing proliferative lymphomatous disease in multiple sites is considered to have only one tumor of this type. Similarly, multiple metastatic lesions are not tabulated or counted as tumors if the primary site is known. One metastatic lesion without a primary site of origin was listed as one tumor under "metastatic tumor, primary site not determined." In addition, endometrial polyps are considered to be tumors for the purposes of this report and are included in this table.

A complete list of the tumors as diagnosed by UAREP is given in Appendix V-21. This table also shows the time of presumed initial observation of each tumor. Two sections that, according to EPL, showed tumors were missing from UAREP's slides and are enumerated in Appendix V-22. In addition, one section is listed as UAREP diagnosis as showing a tumor, whereas EPL denoted it as missing.

Tumor Incidence Statistical Analysis - In Figure No. 7, page 54, of E-70, Hazleton reports the number of histologically proven tumors in male and female albino rats receiving aspartame or serving as controls in the categories which they used for analysis of tumor incidence. In Appendix V-23, UAREP presents the Hazleton data together with the comparable UAREP data based on their diagnoses of the histopathologic material for E-70. UAREP also includes its data for adrenal cortical tumors, adrenal medullary tumors, and pituitary tumors for which Hazleton did not seek to determine tumor incidence. There is generally close, if not always complete, agreement between UAREP and EPL as to the number of tumors in each category analyzed. It should be noted that the category entitled "all benign tumors" shows lower figures for EPL because they counted only animals who had a benign tumor. If a rat had a malignant and a benign tumor, it was counted only under the malignant category. The rationale for the criteria adopted by HLA could be that if the animal had a malignant tumor it was of lesser importance whether it also had a benign tumor. UAREP felt that it was of importance to know whether primarily malignant or primarily benign tumors were being produced and therefore tallies the benign tumors slightly differently than HLA.

In Figure No. 8, presented on page 55 of E-70, Hazleton presents their data for probabilities of tumor incidence in these rats. Their figures are compared with UAREP data in Appendix V-24 based on our review of the histopathologic slides. The values for the probability of tumor incidence are based on the life table analysis method using the final cumulative incidence figures at the termination of the experiment. In general, the UAREP and HLA cumulative incidence agreed completely

until the 90th or 100th week of the experiment. There was significant disagreement as one proceeded beyond the 100 weeks. The tapes provided by HLA for their tumor analysis showed data for 103 weeks, but never for 104 weeks. The 103 week figures appeared to contain at least some of the animals that showed tumors at terminal sacrifice. Because of the consistent difference in terminal values for cumulative incidence by the life table method of analysis, UAREP decided it was not worth further attempts on its part to ascertain what HLA had done differently to achieve its final results. UAREP considers the figures on the analysis of the statistical significance of tumor incidence by the life table method of analysis to be more important than the data on the probability of tumor incidence presented in Appendix V-24. As mentioned in the discussion of Chapter IV, UAREP does not clearly understand the ultimate analysis of the HLA tumor data in which they mention the use of a t-test, but do not supply details as to its application. As mentioned earlier, UAREP's analysis by the life table method indicated no significant difference in the tumor incidence in any of the categories of tumors which it compared.

Non-Neoplastic Diseases - A summary tabular comparison of non-neoplastic lesions by UAREP and EPL was not created as was done for Chapter IV, Appendix V-25, A, B, C, D, since such data gives no indication that matching figures mean matching diagnosis with regard to the same animal being involved or the severity of the lesion. Because such summary data can be misleading, UAREP chose to produce such tables only for E-33,34 and for E-75 and not for E-70 or E-76. The tabulation of significant

discrepancies as shown in Appendix V-25, is felt to be more meaningful for checking agreement of diagnoses. According to the definitions given in Chapter II, about half of these could be classified as major discrepancies with 20% involving problems of differentiating between proliferative hyperplasia and proliferative neoplasms, a distinction which can be bothersome and debatable under some circumstances to pathologists. If there is generally good agreement in diagnoses, the frequency of such diagnoses in various groups would not change from that presented by EPL.

Disease Related to Treatment

UAREP's review of its findings agrees with those of HLA, that there is no evidence of aspartame related lesions demonstrated by the histopathologic findings.

Discrepancies in Following Protocol Design and Correlation of Clinical vs Gross Necropsy vs Microscopic Observations -

Since few significant discrepancies were found in the lengthy time consuming process of correlating the clinical, gross, and microscopic findings in E-33,34, such a study was not attempted in this experiment nor were tables prepared describing specific discrepancies. It was UAREP's overall impression from working on the data that there was good clinical-gross-microscopic correlation in E-70. It also appeared that the protocol was followed specifically in terms of carrying out the procedures at necropsy, and subsequent histopathologic study.

CONCLUSIONS

These experiments involved 280 rats in control and two treatment groups which received 2 gm/kg/day and 4 gm/kg/day of aspartame. The earliest Searle protocol was transmitted December 28, 1972 for experiments which began January 6, 1971 and terminated January 3-12, 1973. There was some confusion regarding chemistry procedures to be carried out at termination. Some of the memoranda relative to this terminal work were dated after the sacrifice of animals had begun. UAREP encountered problems in following the precise statistical methods employed, since they were not presented with clarity in either the protocols or Entry Book report. The problems were the same as those described in Chapter IV.

UAREP's sampling of data on body weight gains and food consumption agreed closely with that presented by Hazleton.

UAREP and Hazleton's life table analysis data showed excellent agreement in cumulative survival rate up to 100 weeks, but there were significant discrepancies in the analysis of the terminal data. UAREP is unable to explain the reasons for these differences.

Six hematologic, 17 clinical chemical, and seven urinalysis parameters were measured. Many of these were measured at four or five intervals throughout the experiment. As in E-33,34, due to deaths and shifting of animals from clinical chemistry to hematology parameter measurements, it was necessary to use many different animals over the course of the experiment instead of the five per group originally intended. The variabilities in hematologic and clinical chemistry results

encountered in E-70 were similar to those encountered in E-33,34. It was not feasible for UAREP to determine the extent to which such variability related to inherent biologic factors in the rats, variations in methods of collecting, transporting, storing, and analyzing the specimens, or to experiment design and other factors.

UAREP's validation of the handling of data showed that transcriptional errors were rare. Rounding discrepancies were inconsequential as were most of the few computational errors discovered. The differences in statistical analysis of the laboratory data related more to the fact that UAREP employed some methods which were different than those used by Hazleton rather than to differences in the results of comparable use of statistical methods. UAREP agreed with the Entry report that the evidence was wanting that the statistically significant variations in findings were compound or dose related.

UAREP's validation of organ and organ to body weight ratios showed a high degree of correlation with those of Hazleton.

Although there were discrepancies in histopathologic diagnoses between EPL and UAREP, it was felt that because of the large number of sections involved, there was generally good agreement. As in the case of E-33,34, UAREP was unable to duplicate precisely the statistical analysis for tumor incidence as carried out by HLA. Of more significance was the fact that UAREP found no statistically significant differences in tumor incidence when comparing all the tumor categories used by HLA as well as several others not used. The only significant difference which HLA found was a decrease in Group 2 as compared with the Control

animals for the incidence of benign tumors.

In UAREP's review of the experiments in E-70, we found no evidence that there were any changes other than decreased food consumption and weight gain in rats consuming large amounts of aspartame. The changes in all the parameters were not considered to be aspartame or dose related.

CHAPTER V

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CHAPTER V

E-70

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APPENDIX V-1

HAZLETON PROJECT SHEETS, SEARLE PROTOCOL AND AMENDMENTS
AND INTERNAL MEMORANDA RELATING TO E-70

Item A - Hazleton Project Sheet No. 1 dated July 15, 1970.

Item B - Searle Protocol and Amendment No. 1 dated December 28, 1972.

Item C - Hazleton Project Sheet No. 2 dated January 10, 1973.

Item D - Hazleton Internal Memorandum dated January 10, 1973.

Item E - HLA Data Sheets for Liver Phenylalanine Hydroxylase.

Comments

The Searle Protocol and Amendment No. 1 (*Item B*) appears to be the first document generated by Searle that was provided to UAREP indicating its protocol design. The typed date is December 28, 1972; the date of Dr. McConnell's signature is January 3, 1973, so apparently its transmittal began one day before the experiments were to be concluded. A copy of the Hazleton Project Sheet No. 1 dated July 15, 1970 was attached to the Searle protocol. It was check-marked as the sponsor's copy. The copy of the same document (*Item A*) supplied to UAREP by Hazleton had crossed out the specifications for weighing of the spleen and urinary bladder and added the requirements for weighing of adrenal, seminal vesicles, prostate, uterus, and tissue masses. The Searle protocol reflected these changes in the initial Hazleton Project Sheet No. 1, except it deleted weighing of tissue masses.

Hazleton Project Sheet No. 2, dated January 10, 1973, implements the requests for protocol change with regard to the collection of liver samples for phenylalanine hydroxylase, urine samples for determining homogentisic acid, and the changes in the necropsy protocol. It does not, however, mention the five serum chemistries to be added. The additional chemistry sample collection for total cholesterol and triglycerides are mentioned in the hand-written, unsigned memorandum to Petrovics dated February 2, 1973 (*Item D*). The papers made available to UAREP contained no documentation of Hazleton's requesting collection of specimens for serum insulin, L-phenylalanine, and L-tyrosine. Results for insulin are reported in E-70, Appendix page 34, along with the triglycerides and total cholesterol on pages 32 and 33. UAREP has two pages of raw data (*Item E*) for determinations for liver phenylalanine hydroxylase which is reported in E-70, Table No. 7A, Appendix page 73. The request specified that the total cholesterol, triglycerides, insulin, and liver phenylalanine hydroxylase be run on the same mice. This was done with the exception that liver phenylalanine hydroxylase was run on female 90897 instead of 90889. The animal numbers are correctly identified in the E-70 report. The serum L-phenylalanine and L-tyrosine were to be run at Searle Laboratories, but UAREP has no information regarding the samples or determinations and they are not mentioned in the E-70 report. The number of the animal numbers which were changed on these two sheets of *Item E* were initialed. The animals under Group 4 with dosage of 5 mg/kg/day and animal 90322 are from some other experiment.

It is of interest that a number of the internal memoranda of Hazleton to carry out procedures on the rat at the termination of the experiment were dated on January 10 and February 2, whereas the experiment was scheduled for termination and rats were actually sacrificed on January 3, 5, and 9-12, 1973. It is possible that there were undocumented oral communications between Searle and HLA staff prior to the written memos.

Initiates

1-6-71

HAZLETON LABORATORIES PROJECT SHEET

850755

PROJECT SHEET NO. <u>1</u>		PROJECT NO. <u>700-240</u>	
PROJECT COORDINATOR <u>Jessup/Reno</u>		DATE <u>July 15, 1970</u>	
COMPOUND(S) <u>SC-18862</u>	LOT NO(S).	RECEIPT DATE <u>7-9-70</u>	LH-NUMBER(S) <u>12,237K</u>
DIVISIONS PARTICIPATING <u>Toxicology</u>	DISTRIBUTION: CENTRAL FILE (2) EACH DIV. PARTICIPATING EACH DIV. DIRECTOR		
SPONSOR PROJ. COORD. DATA PROCESSING			
PHYSICAL AND CHEMICAL PROPERTIES			
SPECIAL PRECAUTIONS (INDICATE PERSONNEL HAZARDS, PROTECTIVE INSTRUCTIONS)			
REFERENCE INFORMATION			
PROGRESS REPORTS DUE	FINAL REPT DUE	INITIALS	SIGNATURE
	<u>on completion</u>	<u>DCJ:mcs</u>	<u>[Signature]</u> COORDINATOR

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JUL 16 1970

CHRONIC TOXICOLOGY SECTION

EXPERIMENTAL WORK to be performed in Small Animal Toxicology Section

Lifetime Toxicity Study - Rats

Objective - The purpose of this study is to evaluate the toxicity of SC-18862 in rats which have been derived from parents which received compound during pregnancy and weaning. The offspring will then continue on treatment for two years more.

Animal Groups - The rats for this study will be selected from the F₁ litters produced in the Two-Generation Reproduction Study, Hazleton Laboratories Project No. 700-239; and these animals will be randomly divided into the following groups:

Group No.	No. of Animals		Dose Levels gm/kg
	male	female	
1 (Control)	60	60	0
2	40	40	2
3	40	40	4
	140	140	180

Group No. 1 will serve as a control group and will be treated in the same manner as the other groups, except that no test material will be administered.

The rats, after weaning, will be individually housed.

Water and the appropriate diets will be freely available during the course of the study.

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9/1/70

Project Sheet No. 1
Project No. 700-240

- 2 -

July 15, 1970

Diet Preparation - The basal laboratory diet will consist of a commercial ration. The test material will be incorporated into the basal diet on a weight-per-weight basis and mixed in a twin-shell blender to provide the appropriate dietary level for each group. Fresh diets will be prepared every week.

Observations - Individual body weights and food consumption will be recorded weekly for the first 26 weeks, biweekly for the succeeding 26 weeks, and once every four weeks thereafter.

Observations of gross signs of toxicity; pharmacological effects; and the incidence, size, and location of tumors will be recorded at the same intervals.

The rats will be observed daily for mortality.

Necropsies - Necropsies will be performed on all rats which die during the course of the study, and tissues will be taken.

Ophthalmoscopic Examination - Ophthalmoscopic examination will be performed on all animals prior to initiation, at one year, and at termination.

Clinical Studies - The following clinical studies will be performed on five male and five female animals from the control and each test group:

Hematology - At six, 13, 26, 52, and 104 weeks:

hematocrit
hemoglobin
erythrocyte count

total leukocyte count
differential leukocyte count

Clinical Biochemistry - At six, 13, 26, and 52 weeks:

fasting blood sugar
blood urea nitrogen
total serum protein
total serum bilirubin

serum glutamic-pyruvic transaminase
serum alkaline phosphatase
serum electrophoresis

- At 104 weeks:

fasting blood sugar
blood urea nitrogen
total serum protein
total serum bilirubin
serum albumin
serum sodium
serum potassium

carbon dioxide
serum calcium
serum chloride
serum glutamic-pyruvic transaminase
serum alkaline phosphatase
serum glutamic-oxaloacetic transaminase
serum electrophoresis

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Project Sheet No. 1
Project No. 700-240

- 3 -

July 15, 1970

Urine Analyses - At six, 13, 26, 52, and 104 weeks (^{IND.} pooled samples):

pH
specific gravity
glucose
ketones

total protein
bilirubin
microscopic examination of
sediment

- At monthly intervals throughout the study, phenylketonuria (dipstick method) will be taken.

Terminal Necropsy - At 104 weeks, the study will be terminated; and the following procedures will be followed (see attachment).

Analysis and Report - At termination of the study, the results will be reported in full giving:

experimental design
general physical
appearance
behavior
effects on body weight, food
consumption, and survival

gross signs of toxic or pharmacologic
effects
clinical findings
individual gross and microscopic
necropsy findings

- Statistical evaluation:

body weights
food consumption
survival

organ weights
organ/body weight ratios

- Tables will be furnished showing:

mean weekly body weights
weight ranges
food consumption
survival data
individual hematological
values

individual biochemical values
results of urine analysis
mean terminal body weights, organ
weights, and organ/body weight
ratios
tissue mass incidence

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July 15, 1970

Organ Weights - Indicated in Column I from each animal.

Histopathological Evaluation - From 5 males and 5 females in Groups No. 1 and No. 3.

- From 5 males and 5 females in Group No. 2 :

Tissues	Histopathology.						Special Observations or Procedures		
	I Wgt.	II Fixed	Group No.						
			1	2	3	4		5	6
Brain		X							
Pituitary		X	X		X				
Spinal Cord		X							
Eye		X							
Salivary Gland		X							
Thyroid	X	X	X	X	X				
Parathyroid									
Thymus									
Trachea									
Esophagus									
Lung		X							
Heart	X	X	X		X				
Liver	X	X	X	X	X				
Pancreas		X	X		X				
Spleen		X	X		X				
Kidney	X	X	X	X	X				
Adrenal	X	X	X		X				
Stomach		X	X	X	X				
Pancreas		X							

APPENDIX V-1

Item B

5109

December 28, 1972

MEMO TO: SC-18862 Preclinical Safety Studies Protocol Design Committee.

Dr. Dutt (Biostatistician) Dr. Ranney (Drug Metab. Rep.)
Dr. F. Saunders (Bio. Res. Dir.) Dr. Polk (Clinical Rep.)

COPY TO: Dr. Reno; HLI (Gen'l Tox. Lab.)

FROM: Dr. Rao (P-T Dept. Monitor)
✓ Dr. McConnell (P-T Dept. Advisor)

SUBJECT: SC-18862: Lifetime Toxicity Study in the Rat; P-T 892H72:
Protocol Amendment No. 1.

The following terminal (104 week) alterations to the above study protocol are incorporated:

1. Clinical chemistry. Measure the following additional parameters terminally from the same animals employed for other clinical chemistry measurements.
 - *a. serum L-phenylalanine
 - *b. serum L-tyrosine
 - c. serum total cholesterol
 - d. serum triglycerides
 - e. serum insulin
2. Liver phenylalanine hydroxylase. To be evaluated from the same animals employed for clinical chemistry measurements. The detailed procedure is enclosed. (For more information on the method contact Dr. Radzialowski of Searle Laboratories; 312 - 673-3200 Ext. 2162). Do same animals as in item 1, above.
3. Revised necropsy protocol enclosed. (procedure attached).
4. Urinalysis. Urinary Homogentisic acid should be measured from the same animals employed for other clinical chemistry measurements. Do control and high dose groups; if latter negative, do no more. Method attached.

* To be performed at Searle Laboratories. Requires 0.5 ml of frozen serum for combined items a & b.

1100

SC-18862 Design Committee
December 28, 1972
Page 2

The original protocol (attached; Hazleton Labs format; 7-15-70) has been reduced to the current format employed by Searle Labs, and a copy is attached. Addition of Amendment No. 1 thus provides a complete, current protocol.

R. S. Rao, Ph. D.
K. S. Rao, Ph. D.

R. G. McConnell, Ph. D.
R. G. McConnell, Ph. D.

1-353

KSR/RGMC:dv

Attachments (3)

SC-18862: LIFETIME ORAL TOXICITY STUDY IN THE RAT; P-T No. 392H72

Attachment: Protocol Amendment No. 1

Phenylalanine Hydroxylation

(Liver Phenylalanine Hydroxylase)

(110)

REAGENTS AND SOLVENTS

1. 0.05M Tris buffer (pH 6.8) used in homogenate and incubation mixtures (see sheet on Buffers and Solutions)
2. Cofactor solution - DPN (4 μ mole), nicotinamide (20 μ mole), catalase (2,000 units), pterine (0.2 μ mole), IN 0.05M TRIS BUFFER (pH 6.8)
3. L-Tyrosine (1 μ mol/ml)
4. L-Phenyl-Alanine (2 μ mol/ml)
5. Trichloroacetic acid (20%)
6. 1-Nitroso-2-naphthol - 0.1% in 95% methanol
7. Nitric acid reagent - 1:5 nitric acid containing 0.5 mg/ml sodium nitrite
8. Methylene chloride

PROCEDURE

1. Prepare incubation mixtures in disposable plastic 30 x 70 mm vials kept in ice:

<u>Blank*</u>	<u>Standard*</u>	<u>Sample⁺</u>
0.5 ml 0.05M Tris Buffer (pH 6.8)	0.5 ml 0.05M Tris Buffer (pH 6.8)	0.5 ml 0.05M Tris Buffer (pH 6.8)
0.5 ml cofactor	0.5 ml cofactor	0.5 ml cofactor
0.5 ml 10000 ^{16,000} xg super-natant	0.5 ml 10000 ^{16,000} xg super-natant	0.5 ml 10000 ^{16,000} xg super-natant
0.5 ml H ₂ O	0.5 ml L-tyrosine	0.5 ml L-phenyl-alanine

* Immediately after adding all components to blank and standard vials, stop reaction by adding 1 ml trichloroacetic acid 20% and do not incubate

+ Pre-incubate samples 1 minute without L-phenyl-alanine, then add L-phenyl-alanine and incubate sample mixtures with shaking at 37°C for 20 minutes at speed of 100-120 cycles per minute under an atmosphere of oxygen (95%) and carbon dioxide (5%).

Page 2; Phenylalanine Hydroxylase Method

8111

2. After incubation stop reaction with 1 ml 20% trichloroacetic acid and mixing.
3. Transfer incubation mixtures to 16 x 100 mm disposable tubes and centrifuge for 15 minutes at high speed.
4. Transfer 2 ml of clear supernatant fraction to 50 ml screw top tubes. Add 1 ml 1-nitroso-2-naphthol and 1 ml nitric acid reagent.
5. Place tubes in H₂O bath at 55°C for 30 minutes. Stopper tubes.
6. Cool tubes. Add 10 ml methylene chloride to each tube and shake on automatic shaker for 15 minutes.
7. Centrifuge at low speed 5 minutes.
8. Read optical density on upper phase at 450 mμ in the Beckman DB spectrophotometer.

SC-18862: LIFETIME ORAL TOXICITY STUDY IN THE RAT; P-T No. 3921172

6112

Attachment; Protocol Amendment No. 1

Revised Postmortem Procedures

TISSUES	A Wt.	B Fix	C (Micro)			
			L	M	H	C
Stomach		X	40		40	60
Small intestine		X	40		40	60
Large intestine		X	40		40	60
Lung		X	40		40	60
Heart	X	X	40		40	60
Kidney	X	X	40		40	60
Liver	X	X	40		40	60
Spleen		X	40		40	60
Pancreas		X	40		40	60
Pituitary	X	X	40		40	60
Thyroid	X	X	40		40	60
Adrenal	X	X	40		40	60
Gonad	X	X	40		40	60
Uterus/sem.v.	X	X	40		40	60
Vagina/prostate V.	M	X	40		40	60
Mammary gland; R 4&5		X	40		40	60
Brain (2 levels)		X	40		40	60
Spinal cord Cerv.		X	---		40	60
Nerve (brachial plexus)		X	---		40	60
Eye, R.		X	---		40	60
Urinary bladder		X	40		40	60
Salivary gland		X	40		40	60
Lymph node, Mesent.		X	---		40	60
Thymus			---		---	---
Bone marrow Fem. Plug		X	---		40	40
Rib junction		X	---		40	40
Skin		X	---		---	---
Unusual lesions		X	40		40	60
Usual lesions		X	40		40	60

A -- The organs weighed from each animal.

B -- The tissues preserved from each animal.

C -- Tissues examined microscopically from the indicated no. of animals of each sex.

Additional postmortem procedures:

1) Examine all listed tissues from all non-survivors, also, with a concerted attempt to identify the probable cause of death.

2) Freeze liver specimens as necessary for liver phenylalanine hydroxylase measurements requested.

3) Urinary bladder fixation. Remove urine as necessary. Inflate moderately with fixative. Hemisect fixed bladder and examine grossly. Embed both halves and examine two "step" sections (100 μ spread \pm) of each half from all grossly normal bladders.

SC-18862: LIFETIME ORAL TOXICITY STUDY IN THE RAT; P-T 892H72

0133

Attachment; Protocol Amendment No. 1

Urinary Homogentisic Acid

Urine (or blood) normally contains no homogentisic acid (HGA),
a qualitative test would probably suffice:

Add 5.0 ml of 3% (w/v) AgNO_3 to 0.50 ml urine, followed by a few drops of 10% (v/v) NH_4OH . If HGA is present, a brown-black to black precipitate of reduced elemental silver will be formed immediately - often before the addition of the NH_4OH .

Although I have not seen either reference, two are shown below:

Seegmiller, J. E., Zannoni, V. G., Paster, L., and LaDu, B. N. (1961).
An enzymatic spectrophotometric method for the determination of homogentisic acid in plasma and urine. J. Biol. Chem. 236, p. 774.

Sommerfelt, S. C., and Wijnstroot, E. (1957). Detection and rough estimation of homogentisic acid in urine. Scan. J. Lab. Clin. Invest. 9, p. 196.

FINAL PROTOCOL FOR A PRECLINICAL SAFETY STUDY OF SC-18862

COMMERCIAL LAB PROJ. NO. _____ AMENDED ① 2 3 4 5 PATH-TOX PROJ. NO. 892H72

- 1) Protocol finalized 7-15-70 Treatment initiated 1-6-71 Animals terminated 1-3-72 Final report 4-6-73
- 2) Cpd. needed (kg): Total _____ First 4 wks. _____ Ordered _____ Del'vy _____
- 3) Study title & objectives: SC-18862: Lifetime Oral Toxicity Study in the Rat: P-T 892H72.
An evaluation of safety during administration to rats, to support marketing of SC-18862 as
a food additive in the UK, USA, Canada and Europe.
- 4) Species, strain, sex, (M,F): Rat; CRcd; M,F Age (wk) at Rx start: 3 weeks*
- 5) Rx duration (wks): 104 plus* Route & Freq. of admin.: Oral; continuous, ad lib.
- 6) Mode of admin.: Compound admixed (w/w) in diet.
- 7) Drug-vehicle mixture stability analysis; Rx wks.: Stable in animal diets at room temperature.
- 8) Est. daily human (maximal) dose & route: 30 mg/kg (for 27 kg child) orally in divided doses.
- 9) Dose levels (GPK daily): Control 0; Low 2; Med. --; High 4
- 10) Multiple of human dose: --; 67; --; 133
- 11) No. & sex of animals/level; 60 M; 40 M; -- M; 40 M
60 F; 40 F; -- F; 40 F
- 12) Total animals required: 280
- 13) Housing & basal diet: Individual: Rockland Rat/Mouse Complete Diet (meal form):
water ad lib.
- 14) General observations (frequency; wks)
Morbidity-mortality: Observe daily and record.
Motor & behavioral activity: Daily; summarize weekly
Body weight: Weekly up to 26 weeks; bi-weekly for the next 26 weeks and once every 4 weeks thereafter
Food consumption & dose adjust.: Concurrent with body weight interval.
Additional observations: Record pertinent observations.
- 15) Physical examination (frequency; wks)
Gen'l external features, incl. body orifices & excrement: Concurrent with body weight interval.
Limited neurological: -- Detailed neurological: --
Ophthalmoscopic and/or slit lamp: Parental generation - pre Rx; F₁ generation - weaning, 52 wks & at termination
Digital palpation for protruding tissue masses: Once every 4 weeks.
Body temperature (rectal): --
Blood pressure and/or ECG: --

* See page 1A.

Animals employed in this study are the F1A generation from P-T No. 867H71; parents received compound prior to mating, and throughout gestation and lactation. Offspring had compound-diet available to them continuously from birth. Litters were culled to 10 pups; 8.5 pups/litter were available for assignment to this study. Since 20 litters per group were available (170 pups), 80 weanlings (40 M, 40 F) were selected (4 per litter) per treatment group, and 120 weanlings (60 M, 60 F) were selected (6 per litter) for the control group. Thus, protocol design precludes random distribution of pups based on genetic constitution. The F₀ generation (parents) was from a random-outbred colony, however.

Page 2

PROTOCOL FOR A PRECLINICAL SAFETY STUDY OF SC-18882

16-18:

PATH-TOX. PROJ. NO. 892H72

CLINICAL LABORATORY PROCEDURES*

(On F₁ generation only)

0110

Specimen collection: individual
pooled (____/sex/level)

Blood: Unepettes for hematology; serum for clinical chemistry.

Urine: Collected from metabolic changes.

16. HEMATOLOGY

Parameter	No./sex/ level	Rx interval (wks)
Hematocrit.....	5	6,13,26,52,104
Hemoglobin.....	5	6,13,26,52,104
Total RBC.....	5	6,13,26,52,104
Total WBC.....	5	6,13,26,52,104
Differential.....	5	6,13,26,52,104
Reticulocyte.....		
Platelets.....		
Coagulation (L-W).		
Pro. time.....		
Activ. PTT.....		
arrow smear.....		
.....		
.....		

17. URINALYSIS

Parameter	No./sex/ level	Rx interval (wks)
Sp. gravity.....	5	13,26,52,104
Bili-Labstix.....		
pH, Bilirubin, Protein, Sugar, Ketones, Blood.	5	13,26,52,104
Urobilinogen.....		
Microscopic.....	5	13,26,52,104
Phenylketones.... (Phenistix)	5	monthly

18. CLINICAL CHEMISTRY

Parameter	No./sex/ level	Rx interval (wks)	Parameter	No./sex/ level	Rx interval (wks)
BUN.....	5	6,13,26,52,104	GPT.....	5	6,13,26,52,104
Uric acid.....			GOT.....	5	104
Glucose.....	5	6,13,26,52,104	AP.....	5	6,13,26,52,104
Sodium.....	5	104	BSP.....		
Potassium.....	5	104	Bilirubin.....	5	6,13,26,52,104
Calcium.....	5	104	OCT.....		
Fibrinogen.....			CPK.....		
Total Protein.....	5	6,13,26,52,104	Chloride.....	5	104
Protein Electrophoresis	5	6,13,26,52,104			

* Report actual pre-Rx specimen collection(s) as negative number (wks). Clin. lab
workup done preferably on those animals receiving complete postmortem workup.

The usual page 3 of Searle Protocol which contains item 19 on Pharmacological Effects and item 20 on Postmortem Procedures was not included in materials sent from Searle to UAREP. Their pagination stamp shows 0116 on page two of their protocol (page 508) and 0117 on page four (page 509).

Similar information in the same format is shown on page 516 as part of Hazleton protocol amendment No. 1 which was supplied by Hazleton.

Page 4

PROTOCOL FOR A PERCUTANEOUS SAFETY STUDY OF SC-18892

PATH-TOX PROJ. NO. 892H72

21) STATISTICAL EVALUATION OF DATA: PROCEDURES USED

a) Body wt. change; food & drug consumption:

Group mean \pm S. E., appropriate analysis of intergroup variance at each time interval

b) Clinical laboratory values:

Group mean \pm S. E., appropriate analysis of intergroup variance at each time interval

c) Incidence and onset of neoplasms:

Mean incidence and appropriate analysis of intergroup variation at termination, using an actuarial (lifetime) method as employed in P-T No. 83SH71 (700-233)..

d) Randomization procedures:

Simple randomization (see page 1A).

22) INTERIM AND FINAL STUDY REPORTS

The sponsor (Director; Path-Tox Dept) requires a brief quarterly report relating statistically significant changes in items 16, 17, and 18 with a general statement on items 14, 15 and 20, by or on the 1st of Jan., April, July, and October; serious adverse findings are to be reported immediately.

Protocol Distribution List

Design Committee Members:

- | | |
|---------------------------|-----------------------|
| 1) <u>Dr. Dutt</u> | (Biostatistician) |
| 2) <u>Dr. E. Saunders</u> | (Biol. Res. Dir.) |
| 3) <u>Dr. Ranney</u> | (Drug Metab. Reor.) |
| 4) <u>Dr. Polk</u> | (Clinical represent.) |
| 5) <u>Dr. Rao</u> | (P-T Dept. monitor) |
| 6) <u>Dr. McConnell</u> | (P-T Dept. adviser) |


Technical Staff:

- | | |
|-------------------------|------------------|
| 1) _____ | (Path. Lab) |
| 2) _____ | (Autopsy Lab) |
| 3) _____ | (Bio-Anal. Lab) |
| 4) <u>Dr. Reno; HLI</u> | (Gen'l Tox. Lab) |
| 5) _____ | (Hematology Lab) |
| 6) _____ | (Pathologist) |

6220 P-T 847416

HAZLETON LABORATORIES PROJECT SHEET

850755

PROJECT SHEET NO. <u>1</u>		PROJECT NO. <u>700-240</u>																					
		PROJECT COORDINATOR Jessup/Reno	DATE July 15, 1970																				
COMPOUND(S) SC-18862	LOT NO(S).	RECEIPT DATE 7-9-70	LH-NUMBER(S) 12,237K																				
DIVISIONS PARTICIPATING Toxicology	DISTRIBUTION: CENTRAL FILE (2) EACH DIV. PARTICIPATING EACH DIV. DIRECTOR																						
Sponsor <input checked="" type="checkbox"/> PROJ. COORD. DATA PROCESSING																							
PHYSICAL AND CHEMICAL PROPERTIES																							
SPECIAL PRECAUTIONS (INDICATE PERSONNEL HAZARDS, PROTECTIVE INSTRUCTIONS)																							
REFERENCE INFORMATION																							
PROGRESS REPORTS DUE	FINAL REPT DUE on completion	INITIALS DCJ:mtg	SIGNATURE OF PROJECT COORDINATOR 																				
EXPERIMENTAL WORK to be performed in Small Animal Toxicology Section																							
<u>Lifetime Toxicity Study - Rats</u>																							
<p>Objective - The purpose of this study is to evaluate the toxicity of SC-18862 in rats which have been derived from parents which received compound during pregnancy and weaning. The offspring will then continue on treatment for two years more.</p> <p>Animal Groups - The rats for this study will be selected from the F_{1A} litters produced in the Two-Generation Reproduction Study, Hazleton Laboratories Project No. 700-239; and these animals will be randomly divided into the following groups:</p> <table border="1"> <thead> <tr> <th>Group No.</th> <th colspan="2">No. of Animals</th> <th>Dose Levels</th> </tr> <tr> <th></th> <th>male</th> <th>female</th> <th>gm/kg</th> </tr> </thead> <tbody> <tr> <td>1 (Control)</td> <td>60</td> <td>60</td> <td>0</td> </tr> <tr> <td>2</td> <td>40</td> <td>40</td> <td>2</td> </tr> <tr> <td>3</td> <td>40</td> <td>40</td> <td>4</td> </tr> </tbody> </table> <p>Group No. 1 will serve as a control group and will be treated in the same manner as the other groups, except that no test material will be administered.</p> <p>The rats, after weaning, will be individually housed.</p> <p>Water and the appropriate diets will be freely available during the course of the study.</p>				Group No.	No. of Animals		Dose Levels		male	female	gm/kg	1 (Control)	60	60	0	2	40	40	2	3	40	40	4
Group No.	No. of Animals		Dose Levels																				
	male	female	gm/kg																				
1 (Control)	60	60	0																				
2	40	40	2																				
3	40	40	4																				

5119

Project Sheet No. 1
Project No. 700-240

- 2 -

July 15, 1970

Diet Preparation - The basal laboratory diet will consist of a commercial ration. The test material will be incorporated into the basal diet on a weight-per-weight basis and mixed in a twin-shell blender to provide the appropriate dietary level for each group. Fresh diets will be prepared every week.

Observations - Individual body weights and food consumption will be recorded weekly for the first 26 weeks, biweekly for the succeeding 26 weeks, and once every four weeks thereafter.

Observations of gross signs of toxicity; pharmacological effects; and the incidence, size, and location of tumors will be recorded at the same intervals.

The rats will be observed daily for mortality.

Necropsies - Necropsies will be performed on all rats which die during the course of the study, and tissues will be taken.

Ophthalmoscopic Examination - Ophthalmoscopic examination will be performed on all animals prior to initiation, at one year, and at termination.

Clinical Studies - The following clinical studies will be performed on five male and five female animals from the control and each test group:

Hematology - At six, 13, 26, 52, and 104 weeks:

hematocrit	total leukocyte count
hemoglobin	differential leukocyte count
erythrocyte count	

Clinical Biochemistry - At six, 13, 26, and 52 weeks:

fasting blood sugar	serum glutamic-pyruvic transaminase
blood urea nitrogen	serum alkaline phosphatase
total serum protein	serum electrophoresis
total serum bilirubin	

- At 104 weeks:

fasting blood sugar	carbon dioxide
blood urea nitrogen	serum calcium
total serum protein	serum chloride
total serum bilirubin	serum glutamic-pyruvic transaminase
serum albumin	serum alkaline phosphatase
serum sodium	serum glutamic-oxaloacetic trans-
serum potassium	aminase
	serum electrophoresis

0120

Project Sheet No. 1
Project No. 700-240

- 3 -

July 15, 1970

Urine Analyses - At six, 13, 26, 52, and 104 weeks (pooled samples):

pH	total protein
specific gravity	bilirubin
glucose	microscopic examination of
ketones	sediment

- At monthly intervals throughout the study, phenylketonuria (dipstick method) will be taken.

Terminal Necropsy - At 104 weeks, the study will be terminated; and the following procedures will be followed (see attachment).

Analysis and Report - At termination of the study, the results will be reported in full giving:

experimental design	gross signs of toxic or pharmacologic effects
general physical appearance	clinical findings
behavior	individual gross and microscopic necropsy findings
effects on body weight, food consumption, and survival	

- Statistical evaluation:

body weights	organ weights
food consumption	organ/body weight ratios
survival	

- Tables will be furnished showing:

mean weekly body weights	individual biochemical values
weight ranges	results of urine analysis
food consumption	mean terminal body weights, organ weights, and organ/body weight ratios
survival data	tissue mass incidence
individual hematological values	

Project Sheet No. 1
Project No. 700-240

- 4 -

July 15, 1970

POSTMORTEM PROCEDURES

Organ Weights - Indicated in Column I from each animal.

Preservation of Tissues - Indicated in Column II, from each animal, in 10% neutral buffered formalin unless indicated otherwise.

Histopathological Evaluation - From 5 males and 5 females in Groups No. 1 and No. 3.

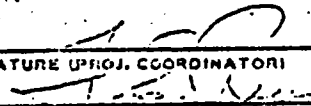
- From 5 males and 5 females in Group No. 2.

Tissues	I Wgt.	II Fixed	Histopathology, Group No.						Special Observations or Procedures
			1	2	3	4	5	6	
Brain		X							
Pituitary		X	X		X				
Spinal Cord		X							
ve		X							
Salivary Gland		X							
Thyroid	X	X	X	X	X				
Parathyroid									
Thymus									
Trachea									
Esophagus									
Lung		X							
Heart	X	X	X		X				
Liver	X	X	X	X	X				
Pancreas		X	X		X				
Spleen	X	X	X		X				
Kidney	X	X	X	X	X				
renal		X	X		X				
Stomach		X	X	X	X				
Pancreas									

B = both sexes; M = male only; F = female only

Item C

HAZLETON LABORATORIES PROJECT SHEET

PROJECT SHEET NO. <u>2</u>		PROJECT NO. <u>700-240</u>	
		PROJECT COORDINATOR <u>Rend Trutter</u>	DATE January 10, 1973
COMPOUND(S) SC-13862		LOT NO(S).	RECEIPT DATE LH-NUMBER(S)
DIVISIONS PARTICIPATING Toxicology-Biochemistry		DISTRIBUTION: CENTRAL FILE (2) EACH DIV. PARTICIPATING EACH DIV. DIRECTOR Sponsor PROJ. COORD. DATA PROCESSING Beaudry	
PHYSICAL AND CHEMICAL PROPERTIES			
SPECIAL PRECAUTIONS (INDICATE PERSONNEL HAZARDS, PROTECTIVE INSTRUCTIONS)			
REFERENCE INFORMATION Letter and protocol from R. G. McConnell - January 3, 1973			
PROGRESS REPORTS DUE	FINAL REPT DUE on compl.	INITIALS FER:da	SIGNATURE (PROJ. COORDINATOR) 
EXPERIMENTAL WORK			
<u>Lifetime Toxicity Study - Rats</u>			
<u>Additions to Protocol</u>			
<u>Histopathology</u> - Revised postmortem procedures are attached.			
<u>Urine Analyses</u> - Urinary homogentisic acid will be determined from urine samples taken from five males and five females in each group prior to necropsy (Clinical Chemistry Section).			
<u>Liver Analyses</u> - Liver phenylalanine hydroxylase activity will be determined from frozen liver samples taken from five males and five females in each group (Biochemistry Department - Beaudry)			
(0000)			

SC-18862: LIFETIME ORAL TOXICITY STUDY IN THE RAT; P-T No. 892H72

Attachment; Protocol Amendment No. 1

Revised Postmortem Procedures

TISSUES	A Wt.	B Fix	C (Micro)			
			L	M	H	C
Stomach		X	40		40	60
Small intestine		X	40		40	60
Large intestine		X	40		40	60
Lung		X	40		40	60
Heart	X	X	40		40	60
Kidney	X	X	40		40	60
Liver	X	X	40		40	60
Spleen		X	40		40	60
Pancreas		X	40		40	60
Pituitary	X	X	40		40	60
Thyroid	X	X	40		40	60
Adrenal	X	X	40		40	60
Gonad	X	X	40		40	60
Uterus/sem.v.	X	X	40		40	60
Vagina/prostate V.	M	X	40		40	60
Mammary gland; R 4&9		X	40		40	60
Brain (2 levels)		X	40		40	60
Spinal cord Cerv.		X	—		40	60
Nerve (cranial plexus)		X	—		40	60
Eye, R.		X	—		40	60
Urinary bladder		X	40		40	60
Salivary gland		X	40		40	60
Lymph node, Mesent.		X	—		40	60
Thymus			—		—	—
Bone marrow Fem. Plus		X	—		40	40
Rib junction		X	—		40	40
Skin		X	—		—	—
Unusual lesions		X	40		40	60
Usual lesions		X	40		40	60

- A — The organs weighed from each animal.
 B — The tissues preserved from each animal.
 C — Tissues examined microscopically from the indicated no. of animals of each sex.

Additional postmortem procedures:

- 1) Examine all listed tissues from all non-survivors, also, with concerted attempt to identify the probable cause of death.
- 2) Freeze liver specimens as necessary for liver phenylalanine hydroxylase measurements requested.
- 3) Urinary bladder fixation. Remove urine as necessary. Inflate moderately with fixative. Hemisect fixed bladder and examine grossly. Embed both halves and examine two "step" sections (100μ spread ±) of each half from all grossly normal bladders.

00007

APPENDIX V-1



HAZLET
a subsidiary of

Item D

DRIES, INC.
Corporation

TO: Heitert

CC: Miller
Petrovics
Trutter

DATE: January 10, 1973

FILE:

SUBJECT: Urine Analyses - Project No. 700-240

FROM: Reno

BLDG.

ROOM:

Attached is a suggested method for the qualitative determination for the presence of homogentisic acid in urine.

This determination should be performed on frozen urine samples from five males and five females on Project No. 700-240 (total of 30 samples).

The urine samples are presently in the custody of Klara Petrovics. Please try to have the determinations completed and the data to me (as well as the cost) before February 2.

FER:dma

(0013

Attachment; Protocol Amendment No. 1

Urinary Homogentisic Acid

Urine (or blood) normally contains no homogentisic acid (HGA),
a qualitative test would probably suffice:

Add 5.0 ml of 3% (w/v) AgNO_3 to 0.50 ml urine, followed by a few drops
of 10% (v/v) NH_4OH . If HGA is present, a brown-black to black precipitate
of reduced elemental silver will be formed immediately - often before the
addition of the NH_4OH .

Although I have not seen either reference, two are shown below:

Seegmiller, J. E., Zannoni, V. G., Paster, L., and LaDu, B. N. (1961).
An enzymatic spectrophotometric method for the determination of homogentisic acid in plasma and urine. J. Biol. Chem. 236, p. 774.

Sommerfelt, S. C., and Wijnstroot, E. (1957). Detection and rough
estimation of homogentisic acid in urine. Scan. J. Lab. Clin. Invest.
9, p. 196.



to Beaudry

cc Stanwick
Truttar
Petrovic

DATE January 10, 1973

FILE

SUBJECT: Liver Analyses - Project No. 700-240

FROM F. E. Reno

BLDG. ROOM

Samples of liver (frozen) from five male and five female rats in each of three groups of animals from this study will require analyses for phenylalanine hydroxylase activity. A copy of the suggested method is attached.

The samples are currently in the custody of Klara Petrovics (Ext. 313), and can be delivered when you are prepared to initiate the analyses.

I will also need an estimate of labor and materials involved in the study.

FER:dma

00013

Phenylalanine Hydroxylase
(Liver Phenylalanine Hydroxylase)

REAGENTS AND SOLUTIONS

1. 0.05M Tris buffer (pH 6.8) used in homogenization and incubation mixtures (see sheet on Buffers and Solutions)
2. Cofactor solution - DPN (4 μ mole), nicotinamide (20 μ mole), catalase (2,000 units), pterine (0.2 μ mole), IN 0.05M TRIS BUFFER (pH 6.8)
3. L-Tyrosine (1 μ mole/ml)
4. L-Phenyl-Alanine (2 μ mole/ml)
5. Trichloroacetic acid (20%)
6. 1-Nitroso-2-naphthol - 0.1% in 95% methanol
7. Nitric acid reagent - 1:5 nitric acid containing 0.5 mg/ml sodium nitrite
8. Methylene chloride

PROCEDURE

1. Prepare incubation mixtures in disposable plastic 30 x 70 mm vials kept in ice:

<u>Blank*</u>	<u>Standard*</u>	<u>Sample⁺</u>
0.5 ml 0.05M Tris Buffer (pH 6.8)	0.5 ml 0.05M Tris Buffer (pH 6.8)	0.5 ml 0.05M Tris Buffer (pH 6.8)
0.5 ml cofactor	0.5 ml cofactor	0.5 ml cofactor
0.5 ml 10000xg supernatant ^{16,000}	0.5 ml 10000xg supernatant ^{16,000}	0.5 ml 10000xg supernatant ^{16,000}
0.5 ml H ₂ O	0.5 ml L-tyrosine	0.5 ml L-phenyl-alanine

- * Immediately after adding all components to blank and standard vials, stop reaction by adding 1 ml trichloroacetic acid 20% and do not incubate
- + Pre-incubate samples 1 minute without L-phenyl-alanine, then add L-phenyl-alanine and incubate sample mixtures with shaking at 37°C for 20 minutes at speed of 100-120 cycles per minute under an atmosphere of oxygen (95%) and carbon dioxide (5%).

CCG16

2. After incubation stop reaction with 1 ml 30% trichloroacetic acid and mix.
3. Transfer incubation mixture to 16 x 100 mm disposable tubes and centrifuge for 15 minutes at high speed.
4. Transfer 2 ml of clear supernatant fraction to 50 ml screw top tubes. Add 1 ml 1-nitroso-2-naphthol and 1 ml nitric acid reagent.
5. Place tubes in H₂O bath at 55°C for 30 minutes. Stopper tubes.
6. Cool tubes. Add 10 ml methylene chloride to each tube and shake on automatic shaker for 15 minutes.
7. Centrifuge at low speed 5 minutes.
8. Read optical density on upper phase at 450 mμ in the Beckman DB spectrophotometer.

CCCC17



HAZLETON LABORATORIES, INC.

a subsidiary of Environmental Sciences Corporation

TO: Petrovics

CC:

DATE: 2/2/73

FILE:

SUBJECT: 700-240

FROM:

BLDG.

ROOM:

- ① Additional clinical chemistry
a) total cholesterol, triglycerides. 1 cc
b) need 0.5 ml frozen serum for shipment to Seattle
c) need 0.7 to 1.0 ml frozen serum for Braudry.
- ② Frozen livers from the 5 males and 5 females used for clinical chemistry.
- ③ need frozen urine on the same 5 c.s. animals - do not pool.

00011

PHENYLALANINE HYDROXYLASE
RAT LIVER

APPENDIX V-1

Item E

$\mu\text{M Tyrosine/gm/}$

GP I	1. ♀	90-888	1-5-73	0.53	✓
	2. ♀	90-893		1.88	-
	4. ♀	90-899 (ST)		1.13	
	1. ♂	90-819		1.25	✓
	2. ♂	90-825		1.67	✓
	4. ♂	90-837		1.09	✓
	3. ♂	90-830		0.94	✓
	5. ♂	90-843 (ST)		1.86	✓
	3. ♀	90-876		0.99	✓
	5. ♀	90-901		1.66	

GP II	♂	90 322	700-246 =	3.82	
	2.	90955		3.85	
	4.	90961		2.81	
	3.	90957		3.12	
	5.	90965		2.91	
	1.	90954		3.36	
	♀ 5	90977 (ST)		1.76	
	43.	90983 (ST)		1.66	
	1.	90987		0.88	
	3.	90992		0.76	
	2.	90990		1.00	

Ap III	♂	4 gm/kg/day	91-016 ¹	3.50
			91-022 ¹	3.32
			91-027 ⁵	5.00
			91-019 ³	1.36
			91-017 ²	2.45
			91-069 ⁴	2.39
			91-056 ¹	1.03
			91-060 ²	2.63
			91-073 ⁵	3.42
			91-062 ³	3.11
	♀			
	♂			
	♂			

Ap IV	♀	5 mg/kg/day	90-947	5.90
	♂		90-453	1.46
	♀		90-490	2.77

WOLF, J and BORY. Biochem. J. 19: 895-913 1955
 UdenFriend, S. and Cooper, J.R. The chemical
 Estimation of Tyrosine and Tyramine J. Biol.
 Chem. 196: 227- 1952,

APPENDIX V-2

PALPABLE NODULES, TISSUE MASSES, AND WART-LIKE LESIONS AS
TABULATED BY UAREP AND HLA (E-34, Page 43)

<u>Group</u>	<u>Males</u>		<u>Females</u>	
	<u>UAREP</u>	<u>HLA</u>	<u>UAREP</u>	<u>HLA</u>
1	10/60	11/60	29/60	29/60
2	8/40	8/40	19/40	22/40
3	8/40	9/40	24/40	24/40

APPENDIX V-2A

SUMMARY OF VARIATIONS AS REPORTED IN CLINICAL OBSERVATIONS MADE
FOR TISSUE MASSES AND NODULES FOR E-70

<u>Group</u>	<u>Animal Number</u>	<u>Variations in Reporting</u>
1F	90877	Nodule reported, week 72-right axilla Nodule not reported, week 76 Abscessed tissue mass reported, week 72-anus Mass not reported, week 88 Mass reported, week 92
1F	90878	Nodule reported, week 60-right axilla Nodule not reported, week 64 Swelling reported, week 60-lower midline Swelling not reported, week 64
1F	90885	Protrusion reported, week 80-both eyes Protrusion not reported, week 84
1F	90886	Nodule reported, week 80-right axilla Nodule not reported, week 84 Nodule reported, week 88
1F	90889	Nodule reported, week 100-right axilla Nodule not reported, week 104
1F	90893	Nodule reported, week 100-right axilla Nodule not reported, week 104
1F	90894	Protrusion reported, week 64-both eyes Protrusion not reported, week 68
1F	90896	Nodule reported, week 84-right inguinal Nodule not reported, week 88

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page 2

<u>Group</u>	<u>Animal Number</u>	<u>Variations in Reporting</u>
1F	90898	Nodule reported, week 60-left axilla Nodule not reported, week 64 Nodule reported, week 60-right axilla Nodule not reported, week 64 Nodule reported, week 60-lower midline Nodule not reported, week 64 Nodule reported, week 84-left inguinal Nodule not reported, week 92 Nodule reported, week 88-right flank Nodule not reported, week 92 Nodule reported, week 88-left flank Nodule not reported, week 92
1F	90902	Protrusion reported, week 92-both eyes Protrusion not reported, week 96
1F	90903	Nodule reported, week 100-left axilla Nodule not reported, week 104 Nodule reported, week 100-right axilla Nodule not reported, week 104
1F	90911	Nodule reported, week 96-left axilla Nodule not reported, week 100 Nodule reported, week 104
1F	90913	Nodule reported, week 88-right flank Nodule not reported, week 92

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page 3

<u>Group</u>	<u>Animal Number</u>	<u>Variations in Reporting</u>
1F	90919	Nodule reported, week 60-left axilla Nodule not reported, week 64
1F	90920	Nodule reported, week 92-right axilla Nodule not reported, week 96 Nodule reported, week 104 Tissue mass reported, week 96-right flank Tissue mass not reported, week 100 Nodule reported, week 100-right side of back Nodule not reported, week 104
1F	90921	Nodule reported, week 88-right flank Nodule not reported, week 92 Nodule reported, week 96 Nodule not reported, week 100 Nodule reported, week 92-right axilla Nodule not reported, week 96 Nodule reported, week 100
1F	90924	Nodule reported, week 92-right flank Nodule not reported, week 96 Nodule reported, week 96-right inguinal Nodule not reported, week 100
1F	90927	Nodule reported, week 60-left inguinal Nodule not reported, week 64

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<u>Group</u>	<u>Animal Number</u>	<u>Variations in Reporting</u>
1F	90927 (cont'd)	Swelling reported, week 88-lower midline Swelling not reported, week 92 Tissue mass reported, week 96 Nodule reported, week 92-anus Nodule not reported, week 96
1M	90819	Swelling reported, week 96-nose Swelling not reported, week 100
1M	90835	Tissue mass reported, week 72-back Tissue mass not reported, week 80 Tissue mass reported, week 84
1M	90858	Nodule reported, week 76-left side of back Nodule not reported, week 80 Nodule reported, week 84-right hind leg Nodule not reported, week 88
2M	90936	Nodule reported, week 100-right axilla Nodule not reported, week 104
2M	90939	Nodule reported, week 25-head Nodule not reported, week 28
2F	90971	Swelling reported, week 88-lower midline Swelling not reported, week 92 Swelling reported, week 96-anus Swelling not reported, week 100

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<u>Group</u>	<u>Animal Number</u>	<u>Variations in Reporting</u>
2F	90975	Nodule reported, week 72-right axilla Nodule not reported, week 88 Tissue mass reported, week 88-right front leg Tissue mass not reported, week 92
2F	90976	Nodule reported, week 60-right axilla Nodule not reported, week 64 Nodule reported, week 96-left axilla Nodule not reported, week 100
2F	90977	Nodule reported, week 60-right axilla Nodule not reported, week 64 Nodule reported, week 68-right axilla
2F	90987	Nodule reported, week 88-left axilla Nodule not reported, week 92
2F	90995	Nodule reported, week 88-left axilla Nodule not reported, week 92 Nodule reported, week 96
2F	91001	Nodule reported, week 36-left inguinal Nodule not reported, week 40
2F	91002	Nodule reported, week 88-left axilla Nodule not reported, week 92
2F	91005	Nodule reported, week 68-mouth Nodule not reported, week 72

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<u>Group</u>	<u>Animal Number</u>	<u>Variations in Reporting</u>
2F	91007	Swelling reported, week 60-lower midline Swelling not reported, week 64 Swelling reported, week 68 Swelling not reported, week 72 Nodule reported, week 68-chest Nodule not reported, week 72 Swelling reported, week 72-anus Swelling not reported, week 76
3M	91021	Nodule reported, week 76-neck Nodule not reported, week 88
3M	91025	Nodule reported, week 56-right axilla Nodule not reported, week 60
3M	91036	Swelling reported, week 96-mouth Swelling not reported, week 100
3F	91052	Nodule reported, week 50-neck Nodule not reported, week 56 Nodule reported, week 60-left axilla Nodule not reported, week 64 Nodule reported, week 68 Nodule not reported, week 72 Nodule reported, week 64-left from paw Nodule not reported, week 68 (cont'd on page 7)

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<u>Group</u>	<u>Animal Number</u>	<u>Variations in Reporting</u>
3F	91052 (cont'd)	Nodule reported, week 64-left flank Nodule not reported, week 68 Tissue mass reported, week 72-left axilla Tissue mass not reported, week 80
3F	91053	Nodule reported, week 100-left axilla Nodule not reported, week 104 Nodule reported, week 100-right axilla Nodule not reported, week 104 Nodule reported, week 100-left inguinal Nodule not reported, week 104
3F	91054	Enlargement reported, week 80-left eye Enlargement not reported, week 88
3F	91055	Nodule reported, week 68-anus Nodule not reported, week 72
3F	91057	Nodule reported, week 76-right ear Nodule not reported, week 80 Nodule reported, week 80-neck Nodule not reported, week 88 Nodule reported, week 100 Tissue mass reported, week 88-chest Tissue mass not reported, week 100
3F	91059	Nodule reported, week 36-right inguinal Nodule not reported, week 38 Nodule reported, week 52-right inguinal

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<u>Group</u>	<u>Animal Number</u>	<u>Variations in Reporting</u>
3F	91060	Nodule reported, week 68-left inguinal Nodule not reported, week 72 Swelling reported, week 88-lower midline Swelling not reported, week 92 Nodule reported, week 96-left axilla Nodule not reported, week 100
3F	91064	Swelling reported, week 60-lower midline Swelling not reported, week 64 Nodule reported, week 72-left axilla Nodule not reported, week 80
3F	91066	Swelling reported, week 88-lower midline Swelling not reported, week 92 Nodule reported, week 92-right inguinal Nodule not reported, week 96 Nodule reported, week 100-right axilla Nodule not reported, week 104
3F	91069	Swelling reported, week 60-lower midline Swelling not reported, week 64 Swelling reported, week 96-mouth Swelling not reported, week 100 Nodule reported, week 100-left inguinal Nodule not reported, week 104

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<u>Group</u>	<u>Animal Number</u>	<u>Variations in Reporting</u>
3F	91074	Nodule reported, week 64-right axilla Nodule not reported, week 68
3F	91078	Nodule reported, week 72-right axilla Nodule not reported, week 76
3F	91083	Swelling reported, week 72-chest Swelling not reported, week 76 Nodule reported, week 76-right axilla Nodule reported, week 80-lower midline Nodule not reported, week 84 Nodule reported, week 88 Nodule not reported, week 96 Nodule reported, week 100
3F	91087	Swelling reported, week 88-chest Swelling not reported, week 92

APPENDIX V-3

STATISTICALLY SIGNIFICANT DIFFERENCES BETWEEN BODY WEIGHTS OF GROUPS AT VARIOUS INTERVALS
BASED ON ANALYSIS OF VARIANCE (ANOVA), LEAST SIGNIFICANT DIFFERENCE
(LSD), AND NEWMAN-KEULS (Q) METHODS AT $P < 0.05$

Interval (week)	Male Groups				Female Groups			
	ANOVA	2<3	1>3	1<2	ANOVA	2<3	1>3	1vs2
0	.68	Ø	Ø	Ø	.65	Ø	Ø	Ø
1	.02	Ø	Ø	+	.20	Ø	Ø	Ø
2	.15	Ø	Ø	Ø	.19	Ø	Ø	Ø
4	.60	Ø	Ø	Ø	.38	Ø	Ø	Ø
8	.02	+	+	Ø	.56	Ø	Ø	Ø
12	.00	+	+	Ø	.58	Ø	Ø	Ø
15	.01	+	+	Ø	.10	Ø	Ø	Ø
20	.01	+	Ø	Ø	.57	Ø	Ø	Ø
24	.01	+	+	Ø	.12	Ø	Ø	Ø
28	.01	+	+	Ø	.21	Ø	Ø	Ø
32	.01	+	+	Ø	.30	Ø	Ø	Ø
36	.02	+	+	Ø	.67	Ø	Ø	Ø
40	.00	+	+	Ø	.11	Ø	Ø	Ø
44	.01	+	+	Ø	.09	Ø	Ø	Ø
48	.01	+	+	Ø	.54	Ø	Ø	Ø
52	.01	+	+	Ø	.11	Ø	Ø	Ø
56	.00	+	+	Ø	.14	Ø	Ø	Ø
60	.08	Ø	Ø	Ø	.31	Ø	Ø	Ø
64	.01	+	Ø	Ø	.46	Ø	Ø	Ø
68	.01	+	Ø	+	.11	Ø	Ø	Ø
72	.28	Ø	Ø	Ø	.33	Ø	Ø	Ø
76	.32	Ø	Ø	Ø	.17	Ø	Ø	Ø
80	.15	Ø	Ø	Ø	.03	Ø	+	Ø
84	.10	Ø	Ø	Ø	.06	Ø	Ø	Ø
88	.38	Ø	Ø	Ø	.05	Ø	±	Ø
92	.53	Ø	Ø	Ø	.02	Ø	+	Ø
96	.22	Ø	Ø	Ø	.01	+	+	Ø
100	.53	Ø	Ø	Ø	.27	Ø	Ø	Ø
104	.65	Ø	Ø	Ø	.28	Ø	Ø	Ø

ANOVA indicates the exact probability that all group means are equal, based upon the F test for Analysis of Variance. UAREP applied the Analysis of Variance at a difference of $P < 0.05$. When values less than 0.05 were obtained the least significant difference (LSD) and Newman-Keuls (Q) tests were run. If the F statistic was greater than 0.05, we did not do the LSD or Q tests because we accept the hypothesis (at the 5% level) that all the means being compared are equal.

Ø means not significant or LSD and Q not done because ANOVA > 0.05 .

+

± means LSD significant, but Q not significant.

APPENDIX V-4A

MALE RATS IN E-70 FROM WHOM BLOOD WAS COLLECTED FOR HEMATOLOGY (H)
OR CLINICAL CHEMISTRY (C) SPECIMENS AT INTERVALS OF 6, 13, 26, 52, & 104 WEEKS

Group	Interval (weeks)				
	6	13	26	52	104
1 Male	90800H →	90808H →	90808H →	90808H →	90808H
	90809H →	90809H →	90809H →	90809H D	90813H
	90810H →	90810H →	90810H →	90810H D	90814H
	90811H →	90811H →	90811H →	90811H D	90816H
	90812H	90812H →	90812H →	90812H →	90812H
1 Male	90813C →	90813C →	90813C →	90813C to H	90819C
	90814C →	90814C →	90814C →	90814C to H	90825C
	90815C →	90815C →	90815C →	90815C D	90830C
	90816C →	90816C →	90816C →	90816C to H	90837C
	90817C →	90817C →	90817C →	90817C D	90843C
2 Male	90928H →	90928H →	90928H →	90928H D	90933H
	90929H →	90929H →	90929H →	90929H D	90934H
	90930H →	90930H →	90930H →	90930H →	90930H
	90931H →	90931H →	90931H →	90931H D	90935H
	90932H →	90932H →	90932H →	90932H →	90932H
2 Male	90933C →	90933C →	90933C →	90933C to H	90954C
	90934C →	90934C →	90934C →	90934C to H	90955C
	90935C →	90935C →	90935C →	90935C to H	90957C
	90936C →	90936C →	90936C →	90936C D	90961C
	90937C →	90937C →	90937C →	90937C D	90965C
3 Male	91008H →	91008H →	91008H →	91008H →	91008H
	91009H →	91009H →	91009H →	91009H D	91013H
	91010H →	91010H →	91010H →	91010H →	91010H
	91011H →	91011H →	91011H →	91011H →	91011H
	91012H →	91012H →	91012H →	91012H →	91012H
3 Male	91013C →	91013C →	91013C →	91013C to H	91019C
	91014C →	91014C →	91014C →	91014C D	91022C
	91015C →	91015C →	91015C →	91015C D	91027C
	91016C →	91016C →	91016C →	91016C →	91016C
	91017C →	91017C →	91017C →	91017C →	91017C

An → indicates same rat used at next interval; rat numbers followed by D, denoting death, or "to H", indicating transfer to hematology tests, were replaced by another rat at the next testing interval.

APPENDIX V-48

FEMALE RATS IN E-70 FROM WHOM BLOOD WAS COLLECTED FOR HEMATOLOGY (H)
OR CLINICAL CHEMISTRY (C) SPECIMENS AT INTERVALS OF 6, 13, 26, 52, AND 104 WEEKS

Group	Interval (week)				
	6	13	26	52	104
1 Female	90874H	90868H	→ 90868H	→ 90868H	→ 90868H
	90870H	→ 90870H	→ 90870H	→ 90870H	D 90875H
	90871H	→ 90871H	→ 90871H	→ 90871H	D 90881H
	90872H	→ 90872H	→ 90872H	→ 90872H	→ 90872H
	90873H	→ 90873H	→ 90873H	→ 90873H	D 90885H
1 Female	90879C	90874C	→ 90874C	→ 90874C	D 90888C
	90875C	→ 90875C	→ 90875C	→ 90875C to H	90889C
	90876C	→ 90876C	90879C	D 90876C	D 90893C
	90877C	→ 90877C	→ 90877C	→ 90877C	D 90896C
	90878C	→ 90878C	→ 90878C	→ 90878C	D 90901C
2 Female	90968H	→ 90968H	D 90973H	→ 90973H	→ 90973H
	90969H	→ 90969H	→ 90969H	→ 90969H	→ 90969H
	90970H	→ 90970H	→ 90970H	→ 90970H	→ 90970H
	90971H	→ 90971H	→ 90971H	→ 90971H	→ 90971H
	90972H	→ 90972H	→ 90972H	→ 90972H	→ 90972H
2 Female	90973C	→ 90973C to H	90978C	→ 90978C	D 90987C
	90974C	→ 90974C	→ 90974C	→ 90974C	D 90990C
	90975C	→ 90975C	→ 90975C	→ 90975C	D 90992C
	90976C	→ 90976C	→ 90976C	→ 90976C	D 90993C
	90977C	→ 90977C	→ 90977C	→ 90977C	D 90997C
3 Female	91048H	→ 91048H	→ 91048H	→ 91048H	→ 91048H
	91049H	→ 91049H	→ 91049H	→ 91049H	→ 91049H
	91050H	→ 91050H	→ 91050H	→ 91050H	D 91053H
	91051H	→ 91051H	→ 91051H	→ 91051H	→ 91051H
	91052H	→ 91052H	→ 91052H	→ 91052H	D 91054H
3 Female	91053C	→ 91053C	→ 91053C	→ 91053C to H	91060C
	91054C	→ 91054C	→ 91054C	→ 91054C to H	91062C
	91055C	→ 91055C	→ 91055C	→ 91055C	D 91069C
	91056C	→ 91056C	→ 91056C	→ 91056C	→ 91056C
	91057C	→ 91057C	→ 91057C	→ 91057C	D 91073C

An → indicates same rat used at next interval; rat numbers followed by D, denoting death, or "to H", indicating transfer to hematology tests, were replaced by another rat at the next testing interval.

APPENDIX V-5

UAREP COMPUTED MEANS AND CONFIDENCE INTERVALS ($P < 0.05$) BASED
ON CONTROL HEMATOLOGY VALUES FOR E-70

Hematocrit

Male Groups

Interval (weeks)	1	2	3	Confidence Interval
6	48	48	49	45 - 51
13	50	50	48	47 - 52
26	48	50	48	46 - 50
52	47	46	47	45 - 48
104	50	45	53	45 - 54

Female Groups

6	48	49	49	46 - 49
13	48	48	47	43 - 52
26	47	45	45	45 - 48
52	44	44	49*	43 - 45
104	44	46	44	39 - 48

Hemoglobin

Male Groups

Interval (weeks)	1	2	3	Confidence Interval
6	15.1	15.3	15.9*	14.5 - 15.6
13	15.9	15.9	14.8	14.8 - 17.0
26	15.5	16.2	15.0	14.8 - 16.2
52	14.7	14.7	15.2	14.1 - 15.2
104	16.6	13.9	17.4	13.5 - 19.6

Female Groups

6	15.6	15.5	15.5	14.9 - 16.4
13	15.2	16.5	17.6*	14.3 - 16.1
26	14.5	14.0	15.0	13.7 - 15.2
52	14.8	14.6	16.0*	14.0 - 15.7
104	14.0	15.3	14.5	12.5 - 15.5

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continued p. 2

RBC

Male Groups

Interval (weeks)	1	2	3	Confidence Interval
6	7.45	7.56	7.64	6.91 - 7.99
13	8.23	8.50	8.28	7.78 - 8.68
26	7.88	8.59*	8.00	7.24 - 8.52
52	7.43	7.93*	7.59	7.02 - 7.84
104	8.63	8.05	9.05	7.54 - 9.72

Female Groups

6	7.42	7.35	7.20	6.96 - 7.88
13	7.65	8.05*	8.33*	7.38 - 7.92
26	7.41	7.55	7.61	6.90 - 7.92
52	7.01	7.01	7.00	6.50 - 7.52
104	7.16	7.50	6.54	6.34 - 7.98

WBC

Male Groups

Interval (weeks)	1	2	3	Confidence Interval
6	21.3	16.1	18.3	13.8 - 28.8
13	28.3	17.0*	20.8*	22.4 - 34.2
26	24.2	16.7	18.1	16.6 - 31.7
52	19.4	16.0	14.0*	14.3 - 24.6
104	18.7	11.8*	11.4*	13.6 - 23.7

Female Groups

6	18.3	12.8	13.5	12.7 - 24.0
13	26.0	14.4*	11.8*	14.8 - 37.1
26	15.8	11.6	10.1	10.0 - 21.6
52	17.0	11.5	10.2	7.0 - 27.1
104	16.3	13.1	11.5*	12.6 - 20.0

* Designates means outside confidence interval

APPENDIX V-6

SUMMARY OF DISCREPANCIES UAREP NOTED IN APPENDIX TABLE NO. 2,
PAGES 10-19, OF ENTRY BOOK E-70

<u>Interval (week)</u>	<u>Para- meter</u>	<u>Group</u>	<u>HLA Value</u>	<u>Type of Discrepancy</u>	<u>UAREP Value</u>
6	Hgb	2M	0.57	R	0.56 (0.5657)
6	RBC	2M	0.55	R	0.54 (0.5453)
6	WBC	2M	3.11	R	3.10 (3.1053)
6	Hgb	3M	S ⁺	ST	See appendix V-7
13	Hgb	3M	S ⁻	ST	See appendix V-7
13	WBC	1F	8.97	R	8.96 (8.9651)
13	Hgb	2F	1.19	R	1.18 (1.1853)
26	RBC	2M	S ⁺	ST	See appendix V-7
26	WBC	2M	S ⁻	ST	See appendix V-7
26	Hct	2M	2.78	R	2.77 (2.7749)
26	WBC	2F	2.99	R	2.98 (2.9853)
52	WBC	1M	4.17	R	4.16 (4.1657)
52	WBC	3M	S ⁻	ST	See appendix V-7

APPENDIX V-7

COMPARISON OF UAREP AND HLA STATISTICALLY SIGNIFICANT
HEMATOLOGY GROUP DIFFERENCES

Parameter	Interval	Sex	ANOVA	Groups	Q	LSD	UAREP t-test	t-test value	HLA t-test
Hct	52	F	.00	1 < 3	S	S	S	4.99	S ⁺
				2 < 3	S	S	S	3.86	ND
	104	M	.03	2 < 3	S	S	S	3.16	ND
Hgb	6	M	.11	1 < 3	ND	ND	N	(2.25)	S ⁺
	13	M	.11	1 > 3	ND	ND	S	2.53	S ⁻
		F	.02	1 < 3	S	S	S	3.26	S ⁺
	26	M	.07	2 > 3	ND	ND	S	2.35	ND
	52	F	.01	1 < 3	S	S	S	3.27	S ⁺
				2 < 3	S	S	S	3.73	ND
	104	M	.06	2 < 3	ND	ND	S	2.69	ND
RBC	26	M	.09	1 < 2	ND	ND	S	2.58	S ⁺
WBC	6	F	.04	1 > 2	N	S	S	2.41	S ⁻
				1 > 3	N	S	N	--	N
	13	M	.01	1 > 2	S	S	S	4.79	S ⁻
				1 > 3	S	S	N	(2.28)	S ⁻
		F	.00	1 > 2	S	S	S	2.79	S ⁻
				1 > 3	S	S	S	3.38	S ⁻
	26	M	.07	1 > 2	ND	ND	N	(2.24)	S ⁻
		F	.04	1 > 3	S	S	S	2.63	S ⁻
	52	M	.07	1 > 3	ND	ND	S	2.41	S ⁻
	104	M	.00	1 > 2	S	S	S	3.45	S ⁻
				1 > 3	S	S	S	3.69	S ⁻
		F	.17	1 > 3	ND	ND	S	2.53	N

ANOVA indicates the exact probability that all group means are equal, based upon the F test for Analysis of Variance. UAREP applied the Analysis of Variance at a difference of $p < 0.05$. When values less than 0.05 were obtained the least significant difference (LSD) and Newman-Keuls (Q) tests were run. If the F statistic was greater than 0.05, we did not do the LSD or Q tests because we accept the hypothesis (at the 5% level) that all the means being compared are equal.

S means $p < 0.05$.

N means $p > 0.05$.

UAREP used the two tailed t-test with 8 degrees of freedom, $t_{0.05} = 2.306$. HLA has not specified their critical value for $t_{0.05}$. They compared experimental groups with controls whereas UAREP did intergroup analysis comparison of all groups.

ND = not done

All ANOVA values of .00 in this report indicate less than 1% chance that means are equal

APPENDIX V-8

DISCREPANCIES UAREP NOTED IN E-70, BLOOD CHEMISTRIES,

APPENDIX TABLE NO. 3, PAGES 20-29

<u>Interval (week)</u>	<u>Parameter</u>	<u>Group</u>	<u>HLA Value</u>	<u>Type of Discrepancy</u>	<u>UAREP Value</u>
26	SGPT	2M	± 4.51	R	4.50 (4.5056)
26	Alk. Phos.	3M	S ⁻	ST	See appendix V-9
52	Glucose	3M	± 6.69	R	6.68 (6.6858)
104	Bilirubin	1F	± 0.217	R	0.218 (0.2175)
104	BUN	3F	S ⁻	ST	See appendix V-9
104	Bilirubin	3F	± 0.019	R	0.020 (0.0195)

All of the inconsequential rounding (R) discrepancies involved standard deviation of means which were correct and would not alter interpretation of results. UAREP and HLA t-test agreed on one statistical (S) discrepancy, but UAREP applied other test which were not significant.

APPENDIX V-9

COMPARISON OF UAREP AND HLA STATISTICALLY SIGNIFICANT GROUP DIFFERENCES
IN CLINICAL CHEMISTRY DETERMINATIONS

Parameter	Inter- val	Sex	ANOVA	Groups	Q	LSD	UAREP t-test	t-test value	HLA t-test
Glucose	52	F	.00	1>2	S	S	S	4.78	S ⁻
				2<3	S	S	S	4.96	ND
BUN	104	M	.02	1<2	S	S	S	2.73	S ⁺
	26	M	.04	1<3	S	S	S	3.41	S ⁺
	104	M	.03	1<2	S	S	N	--	N
				2>3	S	S	S	2.75	ND
SGPT	104	F	.07	1>3	ND	ND	N	(2.24)	S ⁻
		M	.02	1<3	S	S	N	--	N
				2<3	S	S	S	2.79	ND
		M	.07	1>3	ND	ND	S	2.78	S ⁻
Alk. Phos.	13	M	.03	1>3	S	S	S	3.64	S ⁻
				2>3	S	S	N	(2.21)	ND
	26	M	.07	2<3	S	S	S	3.32	ND
				1>3	ND	ND	S	2.78	S ⁻
Bilirubin	104	F	.01	1<2	S	S	S	2.83	S ⁺
				1<3	S	S	S	5.34	S ⁺
Serum Na ⁺	104	M	.00	1<2	N	N	S	4.60	S ⁺
				1<3	S	S	S	3.79	S ⁺
				2<3	N	S	N	(2.16)	ND
Serum K ⁺	104	F	.00	1>2	S	S	S	2.77	S ⁻
				1>3	S	S	S	4.08	S ⁻
Insulin	104	F	.09	1>2	ND	ND	S	2.42	S ⁻

ANOVA indicates the exact probability that all group means are equal, based upon the F test for Analysis of Variance. UAREP applied the Analysis of Variance at a difference of $p < 0.05$. When values less than 0.05 were obtained the least significant difference (LSD) and Newman-Keuls (Q) tests were run. If the F statistic was greater than 0.05, we did not do the LSD or Q tests because we accept the hypothesis (at the 5% level) that all the means being compared are equal.

S means $p < 0.05$.

N means $p > 0.05$.

UAREP used the two tailed t-test with 8 degrees of freedom, $t_{0.05} = 2.306$. HLA has not specified their critical value for $t_{0.05}$. They compared experimental groups with controls whereas UAREP did intergroup analysis comparison of all groups.

ND = not done

APPENDIX V-10

UAREP COMPUTED MEANS AND CONFIDENCE INTERVALS ($p < 0.05$) BASED
ON CONTROL GROUP VALUES FOR CLINICAL CHEMISTRY
PARAMETERS FOR ENTRY BOOK E-70

Total Bilirubin (mg %)

Males

Interval (week)	Groups			Confidence Interval
	1	2	3	
6	0.17	0.16	0.15	0.10 - 0.24
13	0.14	0.14	0.13	0.11 - 0.17
26	0.15	0.16	0.16	0.11 - 0.19
52	0.33	0.28	0.31	0.09 - 0.57
104	0.28	0.29	0.28	0.19 - 0.37

Females

6	0.15	0.15	0.14	0.12 - 0.18
13	0.13	0.13	0.13	0.11 - 0.15
26	0.15	0.16	0.15	0.13 - 0.17
52	0.26	0.23	0.29	0.17 - 0.35
104	0.22	0.28*	0.29*	0.18 - 0.26

Alkaline Phosphatase (K-A units)

Males

Interval (week)	Groups			Confidence Interval
	1	2	3	
6	52.7	47.7	38.9	33.0 - 72.3
13	32.9	30.7	22.0*	26.0 - 39.8
26	33.8	30.5	24.7*	27.0 - 40.6
52	34.7	32.1	26.9	15.7 - 53.7
104	17.5	17.6	18.5	7.3 - 27.6

Females

6	29.4	24.9	34.9	23.0 - 35.9
13	25.5	21.3	30.0	18.3 - 32.8
26	19.8	14.8	19.0	14.3 - 25.3
52	25.1	16.2	22.6	14.5 - 35.8
104	13.1	11.8	18.5*	8.8 - 17.4

Appendix V-10
continued, page two

SGPT (R-F)

Males

Interval (week)	Groups			Confidence Interval
	1	2	3	
6	33	29	34	29 - 37
13	31	36*	35	26 - 35
26	33	35	36	20 - 47
52	41	41	57	25 - 57
104	47	38	71*	35 - 60

Females

6	38	38	45	23 - 53
13	34	39*	40*	32 - 36
26	36	38	38	28 - 44
52	43	70*	51*	38 - 48
104	75	49	62	44 - 107

BUN (mg %)

Males

Interval (week)	Groups			Confidence Interval
	1	2	3	
6	17	16	15	14 - 20
13	15	16	16	14 - 17
26	18	20*	21*	18 - 19
52	17	17	19	15 - 19
104	14	18*	12	10 - 17

Females

6	17	19	19	16 - 19
13	19	20	18	17 - 22
26	23	22	23	21 - 26
52	21	21	24*	18 - 23
104	14	12	10*	11 - 16

Appendix V-10
continued, page three

<u>Glucose (mg %)</u>				
Males				
Interval (week)	Groups			Confidence Interval
	1	2	3	
6	118	111	114	109 - 128
13	119	126	120	108 - 130
26	115	121	126*	108 - 122
52	106	112	115	94 - 119
104	141	195*	160	87 - 194
Females				
6	127	124	128	106 - 149
13	120	113	115	113 - 127
26	118	109*	115	111 - 125
52	113	97*	114	107 - 118
104	148	164	166	109 - 187

* Means falling outside the confidence interval

APPENDIX V-11

UAREP COMPUTED MEANS AND CONFIDENCE INTERVALS ($p < 0.05$) BASED
ON CONTROL GROUP VALUES FOR SERUM Na, K, Ca, Cl,
AND CO₂ PARAMETERS AT 104 WEEKS

<u>Sodium (meq/l)</u>				
<u>Sex</u>	<u>1</u>	<u>Groups</u>		<u>Confidence</u>
		<u>2</u>	<u>3</u>	<u>Intervals</u>
Males	141	145*	151*	138 - 143
Females	144	146*	145	142 - 145

<u>Potassium (meq/l)</u>				
Males	3.7	3.9	4.0	3.2 - 4.2
Females	4.2	3.7	3.5*	3.7 - 4.7

<u>Calcium (mg %)</u>				
Males	10.4	10.1	10.2	9.8 - 11.0
Females	10.3	10.1	10.4	10.0 - 10.6

<u>Chloride (meq/l)</u>				
Males	110	110	110	107 - 114
Females	111	109	108	108 - 114

<u>CO₂ (meq/l)</u>				
Males	23.8	23.3	24.3	22.4 - 25.2
Females	22.6	24.3	24.2	20.3 - 24.3

*Mean values outside confidence interval

APPENDIX V-12

DISCREPANCIES NOTED BY UAREP IN SERUM SODIUM, POTASSIUM, CALCIUM
AND CARBON DIOXIDE RESULTS IN APPENDIX TABLE No. 3,
pp 30-31 OF ENTRY BOOK E-70

<u>Interval</u>	<u>Parameter</u>	<u>Group</u>	<u>HLA Value</u>	<u>Type of Discrepancy</u>	<u>UAREP Value</u>
104	Serum Na	2M	± 0.56	C	0.55 (0.5477)
104	Serum Ca	2M	± 0.47	R	0.46 (0.4658)
104	Serum K	3M	± 0.161	R	0.160 (0.1605)
104	Serum CO ₂	3M	± 1.31	R	1.30 (1.305)
104	Serum Ca	1F	10.27	R	10.28 (10.275)

Four of the five above inconsequential discrepancies
involved standard deviation of means and would not
alter interpretation of results.

APPENDIX V-13

COMPUTED MEANS AND CONFIDENCE INTERVALS ($P < .05$) BASED ON CONTROL GROUPS
FOR PROTEIN AND ELECTROPHORESIS FOR E-70

Total Protein (g%)

Interval (week)	Group	Groups			Confidence Interval
		1	2	3	
6	M	7.98	7.60	8.28	7.36 - 8.60
	F	8.90	7.52*	8.66	7.69 - 10.11
13	M	6.10	6.22	6.30	5.58 - 6.62
	F	6.36	6.30	6.28	5.73 - 6.99
26	M	6.48	6.44	6.94*	6.16 - 6.80
	F	7.02	7.36	8.42*	6.64 - 7.40
52	M	5.50	5.28	5.98*	5.22 - 5.78
	F	5.94	7.06*	6.70*	5.62 - 6.26
104	M	6.66	6.52	6.40	6.14 - 7.18
	F	7.02	6.64*	6.94	6.72 - 7.32

Albumin (%)

Interval (week)	Group	Groups			Confidence Interval
		1	2	3	
6	M	46.0	49.4*	47.2	44.5 - 47.5
	F	46.4	48.9	43.2	37.7 - 55.1
13	M	41.4	43.0	44.1	36.7 - 46.1
	F	47.6	46.4	42.3	40.6 - 54.6
26	M	42.8	42.3	41.1	38.9 - 46.7
	F	49.0	47.9	43.0*	43.4 - 54.6
52	M	41.8	42.8	41.2	36.4 - 47.2
	F	59.2	48.0*	55.2	50.1 - 60.3
104	M	42.8	37.2	41.2	36.0 - 48.3
	F	44.4	40.0*	43.4	40.6 - 48.2

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continued, page two

Alpha 1 (%)					
Interval (week)	Group	Groups			Confidence Interval
		1	2	3	
6	M	17.3	17.9	18.8	14.3 - 20.6
	F	15.4	17.3	16.3	14.0 - 16.8
13	M	20.3	20.7	20.5	18.5 - 22.1
	F	16.4	19.0*	19.9*	14.1 - 18.7
26	M	21.8	20.9	23.6*	20.8 - 22.8
	F	17.6	18.0	17.0	15.4 - 19.8
52	M	21.8	22.4	21.8	19.0 - 24.6
	F	14.0	17.8	13.2	8.2 - 19.8
104	M	17.6	22.0*	17.6	13.6 - 21.6
	F	13.8	15.0	14.4	8.5 - 19.1

<u>Alpha 2 (%)</u>					
Interval (week)	Group	Groups			Confidence Interval
		1	2	3	
6	M	10.6	9.3*	14.0	9.6 - 11.6
	F	14.4	12.0	18.5	4.2 - 24.6
13	M	10.7	10.9	13.6*	9.5 - 11.9
	F	10.6	13.2*	13.2*	9.2 - 12.0
26	M	10.8	11.0	11.7	9.0 - 12.6
	F	10.2	10.3	16.6*	6.3 - 14.1
52	M	11.0	10.4	9.0*	9.2 - 12.8
	F	8.0	5.8*	6.0*	6.2 - 9.8
104	M	13.0	13.0	11.0	9.1 - 16.9
	F	10.8	11.6	10.6	8.8 - 12.8

Interval (week)	Group	Beta (%)			Confidence Interval
		1	2	3	
6	M	20.1	19.0	17.6	17.5 - 22.7
	F	16.4	17.0	16.6	14.5 - 18.3
13	M	22.2	21.2	19.0	18.0 - 26.4
	F	18.6	16.9	17.3	14.8 - 22.4
26	M	20.2	20.6	19.0	16.9 - 23.5
	F	18.2	16.2	17.4	15.4 - 21.0
52	M	20.1	20.0	23.8*	16.6 - 23.6
	F	13.8	22.2*	18.9*	12.0 - 15.6
104	M	15.0	17.8*	20.6*	16.7 - 17.3
	F	18.2	20.6	18.6	13.9 - 22.5

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continued, page three

<u>Gamma (%)</u>					
Interval (week)	Group	Groups			Confidence Interval
		1	2	3	
6	M	6.0	4.4	2.4*	4.2 - 7.8
	F	5.4	4.8	5.2	3.3 - 7.5
13	M	5.2	4.4	3.4*	4.2 - 6.2
	F	6.8	4.5	7.3	3.8 - 9.8
26	M	4.4	5.2	4.6	3.0 - 5.8
	F	5.0	5.6	6.0*	4.1 - 5.9
52	M	5.3	4.4	4.2	2.0 - 8.6
	F	5.0	6.2	6.7	2.5 - 7.5
104	M	11.6	10.0	9.6	8.4 - 14.8
	F	12.8	12.8	13.0	10.8 - 14.8

<u>Total Albumin (g%)</u>					
Interval (week)	Group	Groups			Confidence Interval
		1	2	3	
104	M	2.24	2.22	2.14	1.58 - 2.90
	F	2.68	2.53	2.75	2.22 - 3.14

*Mean values outside confidence interval

APPENDIX V-14

DISCREPANCIES NOTED IN ELECTROPHORESIS DATA IN APPENDIX TABLE
NO. 3, PAGES 35 - 44 OF ENTRY BOOK E-70

Interval (week)	Parameter	Group	HLA Value	Type of Discrepancy	UAREP Value
6	Alpha 2	2M	S ⁻	ST	See Appendix V-15
6	Total Protein	2M	±0.617	R	0.616 (0.6164)
6	Total Protein	2F	S ⁻	ST	See Appendix V-15
13	Gamma	3M	S ⁻	ST	See Appendix V-15
13	Albumin	1F	±5.61	R	5.60 (5.6058)
52	Alpha 2	3F	S ⁻	ST	See Appendix V-15
52	Beta	3M	±9.99	R	9.98 (9.9850)
52	Beta	2F	S ⁺	ST	See Appendix V-15
104	Alpha 2	2M	±2.35	R	2.34 (2.3452)
104	Gamma	2M	±3.61	R	3.60 (3.6056)
104	Total Protein	1F	±0.195	R	0.194 (0.1944)
104	Total Protein	2F	S ⁻	ST	See Appendix V-15
104	Albumin	2F	±2.35	R	2.34 (2.3452)
104	Albumin	2F	S ⁻	ST	See Appendix V-15

All of these seven inconsequential rounding (R) discrepancies involved standard deviations and would not effect interpretation of results. UAREP t-test results agreed with HLA on 124 comparisons of significance at P<0.05. Seven of these which both HLA and UAREP found significant by t-test, UAREP determined them not significant by analysis of variance.

APPENDIX V-15
COMPARISON OF UAREP AND HLA STATISTICALLY SIGNIFICANT GROUP DIFFERENCES
IN BLOOD PROTEIN DETERMINATIONS

Parameter	Inter- val	Sex	ANOVA	Groups	Q	LSD	UAREP t-test	t-test value	HLA t-test
Total Protein	6	F	.08	1<2	ND	ND	S	2.47	S ⁻
	52	F	.02	1<2	S	S	S	4.06	S ⁺
				1<3	S	S	S	2.41	S ⁺
Albumin	104	F	.12	1>2	ND	ND	S	2.89	S ⁻
	52	F	.04	1>2	S	S	S	2.61	S ⁻
	104	F	.30	1>2	ND	ND	S	2.56	S ⁻
Alpha 1	13	F	.05	1<3	S	S	S	2.77	S ⁺
Alpha 2	6	M	.45	1>2	ND	ND	S	2.74	S ⁻
	52	F	.13	1>3	ND	ND	S	2.39	S ⁻
Beta	52	F	.06	1<2	ND	ND	S	2.67	S ⁺
Gamma	6	M	.00	1>2	S	S	N	--	N
				1>3	S	S	S	5.31	S ⁻
				2>3	S	S	S	3.54	ND
	13	M	.07	1>3	ND	ND	S	2.85	S ⁻
		F	.06	2<3	ND	ND	S	3.25	ND

ANOVA indicates the exact probability that all group means are equal, based upon the F test for Analysis of Variance. UAREP applied the Analysis of Variance at a difference of $p < 0.05$. When values less than 0.05 were obtained the least significant difference (LSD) and Newman-Keuls (Q) tests were run. If the F statistic was greater than 0.05, we did not do the LSD or Q tests because we accept the hypothesis (at the 5% level) that all the means being compared are equal.

S means $p < 0.05$.

N means $p > 0.05$.

UAREP used the two tailed t-test with 8 degrees of freedom, $t_{0.05} = 2.306$. HLA has not specified their critical value for $t_{0.05}$. They compared experimental groups with controls whereas UAREP did intergroup analysis comparison of all groups.

ND = not done

APPENDIX V-16

UAREP COMPUTED MEANS AND CONFIDENCE INTERVALS ($P < .05$) FOR CHOLESTEROL,
TRIGLYCERIDES AND INSULIN AT 104 WEEKS

<u>Cholesterol (mg %)</u>				
<u>Sex</u>	<u>Groups</u>			<u>Confidence</u>
	<u>1</u>	<u>2</u>	<u>3</u>	<u>Intervals</u>
Males	149.4	170.0	138.4	65.4 - 233.4
Females	96.4	102.2	127.0*	76.6 - 116.2
<u>Triglycerides (mg %)</u>				
Males	309.0	267.0	168.2	70.4 - 547.6
Females	116.0	143.6	125.0	78.8 - 153.2
<u>Insulin (ng)</u>				
Males	6.32	5.44	4.9	3.42 - 9.20
Females	7.9	5.1	7.5	4.96 - 10.84

APPENDIX V-17

DISCREPANCIES NOTED BY UAREP IN CHOLESTEROL, TRIGLYCERIDE
AND INSULIN ANALYSES FOR 104 WEEK INTERVAL REPORTED
IN APPENDIX TABLE NO. 3, PAGES 32-34, E-70

<u>Parameter</u>	<u>Group</u>	<u>HLA Value</u>	<u>Type of Discrepancy</u>	<u>UAREP Value</u>
Serum Triglyceride	1M	± 193	R	192 (192.57)
	3F	± 29	R	28 (28.5307)
Insulin	1M	5.1	R	5.0 (5.05)
	2M	5.1	R	5.0 (5.05)
	2M	5.9	R	5.8 (5.85)
	2F	S ⁻	ST	See appendix V-9
	2F	4.2	C	5.0 (4.98)
	2F	5.1	C ²	5.2 (5.25)
	2F	± 0.7	C ²	0.5 (0.505)

² Computational error based on previous error.

APPENDIX V-18

COMPARISON OF UAREP AND HLA STATISTICALLY SIGNIFICANT GROUP DIFFERENCES IN LIVER PHENYLALANINE HYDROXYLASE ACTIVITY AT 104 WEEKS IN E-70

Sex	ANOVA	Group	Q	LSD	UAREP t-test	t-test Value	HLA t-test
M	.01	1 < 2	S	S	S	7.25	S ⁺
		1 < 3	S	S	S	2.81	S ⁺
F	.01	2 < 3	S	S	S	2.82	ND
		1 < 3	S	S	S	2.67	S ⁺

ANOVA indicates the exact probability that all group means are equal, based upon the F test for Analysis of Variance. UAREP applied the Analysis of Variance at a difference of $p < 0.05$. When values less than 0.05 were obtained the least significant difference (LSD) and Newman-Keuls (Q) tests were run. If the F statistic was greater than 0.05, we did not do the LSD or Q tests because we accept the hypothesis (at the 5% level) that all the means being compared are equal.

S means $p < 0.05$.

N means $p > 0.05$.

UAREP used the two tailed t-test with 8 degrees of freedom, $t_{0.05} = 2.306$. specified their critical value for $t_{0.05}$. They compared experimental groups with controls whereas UAREP did intergroup comparison of all groups.

ND = not done

APPENDIX V-19
DISCREPANCIES NOTED IN APPENDIX TABLE NO. 7 ORGAN AND
ORGAN/BODY WEIGHT (BW) RATIOS FOR E-70

<u>Group</u>	<u>Parameter</u>	<u>HLA Value</u>	<u>Type of Discrepancy</u>	<u>UAREP Value</u>
2M	Body Weight	509	R	508 (508.5)
3M	Thyroid/BW	.0080%	R	.0081 (.00809)
2M	Kidney Weight	4.51	R	4.52 (4.518)
2M	Kidney Weight	±1.00	R	1.01 (1.008)
2M	Kidney/BW	0.905%	C	0.907 (0.9067)
1M	Adrenals	0.083	R	0.084 (0.0835)

These discrepancies are inconsequential in terms of any effect on interpretation of results. UAREP and HLA agreed in all instances of significance of statistical analysis on these data.

APPENDIX V-19A

COMPARISON OF UAREP AND HLA STATISTICALLY SIGNIFICANT

DIFFERENCES IN ORGAN WEIGHTS AND ORGAN TO

BODY WEIGHT (BW) RATIOS

Parameter	Sex	ANOVA	Groups	Q	LSD	UAREP t-Test	t-Test Value	HLA t-Test
Adrenal	F	.08	1<3	ND	ND	S	2.28	N
Heart	M	.00	1>2	S	S	S	3.02	S ⁻
			1>3	S	S	S	4.13	S ⁻
Liver	F	.02	1<2	N	S	S	2.56	N
			1<3	S	S	S	2.45	N
Kidney	F	.06	1<2	ND	ND	S	2.45	N
Thyroid	F	.00	1<2	S	S	S	3.51	S ⁺
			1<3	N	N	S	2.07	N
Prostate	M	.06	1<3	ND	ND	S	2.48	N
Heart/BW	M	.01	1>2	S	S	S	2.57	S ⁻
			1>3	S	S	S	2.71	S ⁻
Liver/BW	M	.44	1<2	ND	ND	N		S ⁺
			1<3	ND	ND	N		S ⁺
Thyroid/BW	M	.65	1>3	ND	ND	S	6.96	N
Thyroid/BW	F	.01	1<2	S	S	S	3.12	S ⁺
Prostate/BW	M	.00	1<3	S	S	S	3.36	S ⁺

	Male Groups						Female Groups					
	Group 1 UAREP EPL		Group 2 UAREP EPL		Group 3 UAREP EPL		Group 1 UAREP EPL		Group 2 UAREP EPL		Group 3 UAREP EPL	
Adrenal	57	58	38	38	39	40	57	56	38	38	40	38
Cortical adenoma	2	0	0	0	1	1	1	0	2	1	2	1
Pheochromocytoma	5	5	4	5	1	4	0	1	2	3	0	2
Pituitary	57	56	30	37	39	39	56	55	38	37	40	39
Adenoma	10	12	10	11	8	7	27	26	18	17	20	19
Adenocarcinoma	3	0	1	0	0	0	1	1	0	0	3	1
Thyroid	57	58	27	37	39	40	57	56	34	37	36	36
Adenoma	5	6	1	1	*1	0	0	0	0	0	0	0
Carcinoma (C-cell)	1	0	1	0	2	2	1	0	0	0	2	1
Parathyroid adenoma	2	0	1	1	1	2	0	1	2	0	0	1
Liver	59	58	39	40	40	40	58	57	38	39	39	40
Neoplastic nodule/ adenoma	1	0	0	0	1	0	1	0	5	0	5	0
Hepatocellular carcinoma	1	1	1	1	2	2	0	0	0	0	0	0
Adenocarcinoma	0	0	0	0	0	0	0	0	1	2	0	0
Spleen	57	58	39	40	38	38	57	57	38	38	40	40
Hemangioma	0	0	0	0	1	1	0	0	0	0	0	0
Pancreas	56	57	39	40	38	39	59	57	36	38	40	40
Islet cell tumor	3	3	0	0	2	2	1	1	2	2	0	0
Adenocarcinoma	0	0	0	0	0	0	0	2	0	0	0	0
Salivary gland	55	57	37	36	40	40	57	53	35	35	37	57
Adenocarcinoma	0	0	0	0	0	1	0	0	0	0	0	0
Brain	58	60	36	39	40	39	57	57	39	39	40	40
Astrocytoma	3	3	1	1	1	1	1	1	1	1	0	0
Ependymoma	0	0	1	1	0	0	0	0	0	0	0	0
Meningioma	0	0	0	0	0	0	0	0	0	0	0	1
Mammary gland	43	56	29	38	28	37	56	55	39	38	31	38
Fibroadenoma/adenoma	0	0	1	1	3	1	20	15 ^d	12	12(d)	9	9
Adenocarcinoma	1	0	0	0	0	0	2	4	4	3	3	3
Papilloma	0	0	0	0	0	0	0	0	1	0	0	0
Testis	51	59	40	40	24	38						
Mesothelioma	0	0	2	1	0	0	Not applicable					
Prostate	50	58	40	40	27	39						
Squamous cell carcinoma	0	0	0	1	0	0	Not applicable					

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	Male Groups						Female Groups					
	Group 1		Group 2		Group 3		Group 1		Group 2		Group 3	
	UAREP	EPL	UAREP	EPL	UAREP	EPL	UAREP	EPL	UAREP	EPL	UAREP	EPL
Ovary							57	56	37	37	40	40
Granulosa cell tumor							0	0	0	0	2	1
Adenocarcinoma			Not applicable				0	0	0	0	1	1
Neurofibrosarcoma							0	0	0	0	0	1
Uterus							56	54	37	39	39	40
Endometrial polyp							0	0	0	0	1	0
Fibropapilloma			Not applicable				0	0	1	0	0	0
Squamous cell carcinoma							0	0	0	0	0	1
Fibrosarcoma							0	0	1	0	0	0
Urinary Bladder	58	59	40	40	39	39	55	55	38	37	38	39
Fibrous polyp	0	0	0	0	0	0	1	0	0	0	0	0
Skin/Tissue Mass												
Fibroma	1	0	0	0	1	2	1	1	1	0	1	1
Keratoacanthoma	3	3	0	0	3	1	0	0	1	0	1	0
Squamous cell carcinoma	2	2	0	0	0	0	0	0	0	1	0	1
Lipoma	1	0	0	0	1	0	0	0	0	0	0	0
Neurofibrosarcoma/ Sarcoma/Fibrosarcoma	1	2	0	0	2	2	0	0	0	1	1	1
Basal cell carcinoma	0	1	0	0	0	0	0	0	0	0	0	0
Papilloma	0	0	0	0	0	1	0	0	0	0	0	0
Hemangioma	0	0	0	0	0	0	0	0	0	1	0	0
All Organs												
Lymphoma	3	3	1	0	0	1	1	2	2	1	1	2
Metastatic Tumor primary site not determined	0	1	0	0	0	0	3	2	0	0	0	0
Totals (UAREP counts)	48	42	25	24	31	31	61	57	56	45	52	47
Original EPL totals		51		24		32		76		58		52
Average number tumors per animal (UAREP data)	.8	.7	.6	.6	.8	.8	1.0	1.0	1.4	1.1	1.3	1.2

* Diagnosis of "Autolyzed tumor, type undetermined"

d UAREP combined the diagnoses of "Adenoma" and "Fibroadenoma" into one category and counted only 1 such tumor per animal, in order to compare the EPL data with the UAREP data. The following animals were diagnosed by EPL as having both an Adenoma and a Fibroadenoma of the mammary gland, which were counted as one tumor by UAREP: Group 1 Female 90-875 and 90-915, Figure No. 6 page 46 and 48, E-70; Group 2 Female 90-987 and 90-977, Figure No. 6, page 49, E-70

The figures opposite each organ show the number of rats with sections of the organ examined

APPENDIX V-21

RATS WITH TUMORS HISTOLOGICALLY PROVEN BY UAREP
WITH WEEKS OF PRESUMED INITIAL OBSERVATION OF TUMORS

Group	Path No.	Animal No.	Tumor Type	Time (weeks)
1M	71-461	90-808	Adrenal - Pheochromocytoma	104
1M	71-462	90-812	Pituitary - Adenocarcinoma	104
1M	71-465	90-818	Brain - Astrocytoma	104
1M	71-466	90-819	Pituitary - Adenoma	104
			Brain - Astrocytoma	104
1M	71-467	90-825	Adrenal - Pheochromocytoma	104
			Thyroid - Adenoma	104
1M	71-469	90-830	Pituitary - Adenoma	104
1M	71-470	90-832	Mammary gland - Adenocarcinoma	104
			Skin - Fibroma	104
1M	71-471	90-834	Thyroid - C-cell Carcinoma	104
1M	71-472	90-837	Pituitary - Adenoma	104
			Thyroid - Adenoma	104
			Pancreas - Islet Cell Adenoma	104
1M	71-473	90-840	Pituitary - Adenocarcinoma	104
1M	71-475	90-845	Pituitary - Adenoma	104
1M	71-476	90-853	Pituitary - Adenocarcinoma	104
			Thyroid - Adenoma	104
1M	71-478	90-861	Pituitary - Adenoma	104
			Pancreas - Islet Cell Adenoma	104
			Skin - Keratoacanthoma	100
1M	71-479	90-863	Pituitary - Adenoma	104
1M	71-480	90-864	Skin - Keratoacanthoma	72
1M	71-481	90-865	Adrenal - Pheochromocytoma	104
1M	71-482	90-867	Adrenal - Cortical Adenoma	104
1M	71-483	90-836	Pancreas - Islet Cell Adenoma	88
1M	71-484	90-849	Pituitary - Adenoma	101
			Thyroid - Adenoma	101
			Thyroid - Parathyroid Adenoma	101
1M	71-485	90-809	Pituitary - Adenoma	89
1M	71-488	90-815	Adrenal - Cortical Adenoma	33

UAREP recognizes that data on the time of onset of tumors is grossly approximate when using criteria (a) of time of sacrifice or death with proven tumor not previously recognized, or (b) first date of clinical observation of swelling subsequently confirmed histologically as a tumor. Better data is not available.

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Group	Path No.	Animal No.	Tumor Type	Time (weeks)
1M	71-489	90-816	Adrenal - Pheochromocytoma	102
			Pituitary - Adenoma	102
1M	71-491	90-820	Skin - Squamous Cell Carcinoma	96
1M	71-492	90-821	Skin - Lipoma	61
1M	71-498	90-828	Liver - Neoplastic Nodule	96
1M	71-501	90-835	Skin - Keratoacanthoma	72
1M	71-502	90-838	Brain - Astrocytoma	102
1M	71-503	90-839	Adrenal - Pheochromocytoma	103
			Lymphoma	92
1M	71-504	90-841	Thyroid - Parathyroid Adenoma	89
1M	71-506	90-844	Skin (tissue mass) - Fibrosarcoma	100
1M	71-507	90-846	Thyroid - Adenoma	82
1M	71-508	90-847	Pituitary - Adenoma	96
1M	71-512	90-852	Lymphoma	96
1M	71-517	90-858	Liver - Hepatocellular Carcinoma	96
1M	71-519	90-862	Skin - Squamous Cell Carcinoma	76
1M	71-520	90-866	Lymphoma	76
2M	71-561	90-930	Thyroid - C-cell Carcinoma	104
			Testis - Mesothelioma	104
2M	71-562	90-932	Pituitary - Adenoma	104
			Thyroid - Adenoma	104
2M	71-565	90-935	Pituitary - Adenoma	104
2M	71-566	90-936	Mammary gland - Fibroadenoma	100
2M	71-567	90-937	Thyroid - Parathyroid Adenoma	104
2M	71-568	90-938	Liver - Hepatocellular Carcinoma	104
2M	71-571	90-947	Pituitary - Adenocarcinoma	104
2M	71-573	90-951	Lymphoma	104
2M	71-574	90-954	Adrenal - Pheochromocytoma	104
			Pituitary - Adenoma	104
2M	71-576	90-957	Adrenal - Pheochromocytoma	104
2M	71-577	90-960	Pituitary - Adenoma	104
2M	71-578	90-961	Adrenal - Pheochromocytoma	104
			Pituitary - Adenoma	104
2M	71-579	90-962	Pituitary - Adenoma	104
2M	71-580	90-965	Pituitary - Adenoma	104

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Group	Path No.	Animal No.	Tumor Type	Time (weeks)
2H	71-581	90-943	Brain - Ependymoma	54
2H	71-582	90-928	Pituitary - Adenoma	95
2H	71-585	90-939	Pituitary - Adenoma	95
2H	71-594	90-956	Adrenal - Pheochromocytoma	97
			Pituitary - Adenoma	97
2H	71-600	90-967	Brain - Astrocytoma	90
			Testis - Mesothelioma	90
3H	71-523	91-011	Pituitary - Adenoma	104
3H	71-524	91-012	Pituitary - Adenoma	104
			Thyroid - Adenocarcinoma	104
			Skin - Lipoma	104
3H	71-526	91-016	Adrenal - Pheochromocytoma	104
			Thyroid - Parathyroid Adenoma	104
			Brain - Astrocytoma	104
3H	71-527	91-017	Pituitary - Adenoma	104
			Pancreas - Islet Cell Adenoma	104
3H	71-528	91-019	Pancreas - Islet Cell Adenoma	104
3H	71-529	91-022	Skin (tissue mass) - Sarcoma	100
3H	71-536	91-032	Pituitary - Adenoma	104
			Liver - Neoplastic Nodule	104
			Skin - Keratoacanthoma	100
3H	71-537	91-035	Mammary gland - Fibroadenoma	104
3H	71-538	91-036	Liver - Hepatocellular Carcinoma	104
			Mammary gland - Fibroadenoma	104
			Skin - Keratoacanthoma	96
3H	71-539	91-041	Adrenal - Cortical Adenoma	104
			Liver - Hepatocellular Carcinoma	104
3H	71-541	91-046	Pituitary - Adenoma	104
3H	71-542	91-047	Tissue mass - Neurofibrosarcoma	104
3H	71-543	91-014	Spleen - Hemangioma	102
			Pituitary - Adenoma	102
3H	71-544	91-015	Pituitary - Adenoma	87
3H	71-548	91-021	Tissue mass - Fibroma	76
3H	71-550	91-025	Skin - Keratoacanthoma	60
3H	71-551	91-031	Pituitary - Adenoma	103

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Group	Path No.	Animal No.	Tumor Type	Time (weeks)
3M	71-554	91-037	Thyroid - C-cell Carcinoma	82
3M	71-556	91-039	Thyroid - Autolyzed Tumor	65
3M	71-560	91-045	Mammary gland - Fibroadenoma	82
1F	71-601	90-868	Pituitary - Adenoma	104
1F	71-602	90-872	Pituitary - Adenoma	104
			Mammary gland - Fibroadenoma	88
1F	71-603	90-875	Pituitary - Adenoma	104
			Mammary gland - Fibroadenoma	104
			Urinary bladder - Fibrous Polyp	104
1F	71-604	90-881	Pancreas - Islet Cell Adenoma	104
1F	71-605	90-885	Pituitary - Adenoma	104
1F	71-606	90-887	Pituitary - Adenoma	104
			Mammary gland - Adenocarcinoma	104
1F	71-607	90-888	Pituitary - Adenoma	104
			Mammary gland - Fibroadenoma	100
1F	71-609	90-893	Pituitary - Adenoma	104
1F	71-611	90-896	Pituitary - Adenoma	104
			Mammary gland - Fibroadenoma	88
1F	71-612	90-897	Pituitary - Adenoma	104
			Mammary gland - Fibroadenoma	88
1F	71-613	90-900	Pituitary - Adenoma	104
			Mammary gland - Adenocarcinoma	96
1F	71-615	90-903	Pituitary - Adenoma	104
1F	71-619	90-910	Pituitary - Adenoma	104
1F	71-620	90-911	Pituitary - Adenoma	104
			Mammary gland - Fibroadenoma	96
1F	71-621	90-913	Mammary gland - Fibroadenoma	96
1F	71-622	90-916	Mammary gland - Fibroadenoma	104
1F	71-623	90-917	Pituitary - Adenoma	104
			Mammary gland - Fibroadenoma	104
1F	71-624	90-920	Pituitary - Adenoma	104
			Mammary gland - Fibroadenoma	92
			Tissue mass - Fibroma	92
1F	71-625	90-921	Adrenal - Cortical Adenoma	104
			Mammary gland - Fibroadenoma	92

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Group	Path No.	Animal No.	Tumor Type	Time (weeks)
1F	71-627	90-924	Mammary gland - Fibroadenoma	92
1F	71-628	90-925	Pituitary - Adenoma	104
1F	71-629	90-926	Pituitary - Adenoma	104
			Mammary gland - Fibroadenoma	104
1F	71-632	90-871	Mammary gland - Fibroadenoma	96
1F	71-633	90-873	Pituitary - Adenoma	101
			Mammary gland - Fibroadenoma	80
1F	71-634	90-874	Metastatic Tumor, undetermined origin	81
1F	71-635	90-876	Brain - Astrocytoma	85
1F	71-636	90-877	Pituitary - Adenocarcinoma	95
			Thyroid - C-cell Carcinoma	95
			Mammary gland - Fibroadenoma	64
1F	71-637	90-878	Pituitary - Adenoma	95
1F	71-639	90-880	Lymphoma	60
1F	71-640	90-882	Mammary gland - Fibroadenoma	60
1F	71-641	90-883	Pituitary - Adenoma	95
1F	71-642	90-884	Pituitary - Adenoma	91
1F	71-643	90-886	Pituitary - Adenoma	92
			Mammary gland - Fibroadenoma	80
1F	71-646	90-892	Pituitary - Adenoma	77
1F	71-648	90-898	Anaplastic Tumor	95
1F	71-649	90-899	Pituitary - Adenoma	97
1F	71-650	90-902	Pituitary - Adenoma	98
1F	71-656	90-915	Mammary gland - Fibroadenoma	80
1F	71-658	90-919	Pituitary - Adenoma	103
			Liver - Neoplastic Nodule	103
1F	71-659	90-922	Adenocarcinoma of undetermined origin	102
1F	71-660	90-927	Pituitary - Adenoma	96
			Mammary gland - Fibroadenoma	88
2F	71-701	90-969	Liver - Neoplastic Nodule	104
			Brain - Astrocytoma	104
2F	71-702	90-971	Thyroid - Parathyroid Adenoma	104
2F	71-703	90-972	Pituitary - Adenoma	104
			Mammary gland - Adenocarcinoma	104
2F	71-704	90-973	Pituitary - Adenoma	104
2F	71-705	90-976	Mammary gland - Fibroadenoma	104
2F	71-706	90-984	Pituitary - Adenoma	104

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Group	Path No.	Animal No.	Tumor Type	Time (weeks)
2F	71-707	90-987	Pituitary - Adenoma	104
			Liver - Neoplastic Nodule	104
			Mammary gland - Fibroadenoma	88
2F	71-709	90-992	Adrenal - Cortical Adenoma	104
			Pituitary - Adenoma	104
2F	71-710	90-993	Liver - Neoplastic Nodule	104
			Mammary gland - Fibroadenoma	100
2F	71-711	90-995	Pituitary - Adenoma	104
			Pancreas - Islet Cell Adenoma	104
			Mammary gland - Fibroadenoma	88
2F	71-714	91-003	Pituitary Adenoma	104
			Liver - Neoplastic Nodule	104
			Mammary gland - Fibroadenoma	96
2F	71-715	91-005	Adrenal - Cortical Adenoma	104
2F	71-716	91-006	Pituitary - Adenoma	104
			Mammary gland - Adenocarcinoma	104
2F	71-717	90-970	Pituitary - Adenoma	102
			Pancreas - Islet Cell Adenoma	102
			Mammary gland - Fibroadenoma	100
2F	71-718	90-974	Liver - Neoplastic Nodule	87
2F	71-719	90-975	Pituitary - Adenoma	98
			Mammary gland - Fibroadenoma	72
2F	71-720	90-977	Pituitary - Adenoma	102
			Mammary gland - Fibroadenoma	60
2F	71-721	90-978	Pituitary - Adenoma	101
			Thyroid - Parathyroid Adenoma	101
2F	71-723	90-980	Mammary gland - Adenocarcinoma	49
2F	71-724	90-981	Pituitary - Adenoma	95
2F	71-726	90-983	Pituitary - Adenoma	99
2F	71-727	90-985	Liver - Adenocarcinoma	96
			Lymphoma	96
2F	71-728	90-986	Mammary gland - Fibroadenoma	88
2F	71-729	90-988	Pituitary - Adenoma	95
			Skin - Keratoacanthoma	88
2F	71-733	90-996	Mammary gland - Fibroadenoma	76
2F	71-734	90-998	Adrenal - Pheochromocytoma	92
			Pituitary - Adenoma	92
			Liver - Adenocarcinoma	92

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Group	Path No.	Animal No.	Tumor Type	Time (weeks)
2F	71-735	90-999	Uterus - Fibrosarcoma	103
2F	71-736	91-000	Pituitary - Adenoma	96
			Mammary gland - Fibroadenoma	72
			Tissue mass - Fibroma	72
2F	71-737	91-001	Mammary gland - Adenocarcinoma	50
			Lymphoma	73
2F	71-738	91-004	Adrenal - Pheochromocytoma	103
			Mammary gland - Fibroadenoma	100
2F	71-739	91-007	Pituitary - Adenoma	83
			Mammary gland - Papilloma	83
			Uterus - Fibropapilloma	83
3F	71-661	91-048	Ovary - Granulosa Cell Tumor	104
3F	71-662	91-049	Pituitary - Adenoma	104
			Liver - Neoplastic Nodule	104
			Mammary gland - Fibroadenoma	96
3F	71-663	91-051	Mammary gland - Fibroadenoma	56
3F	71-664	91-053	Pituitary - Adenoma	104
			Liver - Neoplastic Nodule	104
			Mammary gland - Fibroadenoma	100
3F	71-665	91-054	Ovary - Adenocarcinoma	104
3F	71-666	91-055	Pituitary - Adenoma	104
3F	71-668	91-057	Pituitary - Adenoma	104
			Mammary gland - Fibroadenoma	88
3F	71-669	91-060	Pituitary - Adenocarcinoma	104
3F	71-670	91-062	Mammary gland - Fibroadenoma	96
3F	71-671	91-063	Pituitary - Adenoma	104
			Ovary - Granulosa Cell Tumor	104
3F	71-672	91-066	Pituitary - Adenoma	104
			Thyroid - C-cell Carcinoma	104
3F	71-673	91-069	Skin - Fibroma	96
3F	71-674	91-073	Pituitary - Adenoma	104
3F	71-675	91-074	Liver - Neoplastic Nodule	104
3F	71-676	91-079	Pituitary - Adenoma	104
			Liver - Neoplastic Nodule	104
			Mammary gland - Adenocarcinoma	88
3F	71-677	91-080	Adrenal - Cortical Adenoma	104
			Pituitary - Adenoma	104

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Group	Path No.	Animal No.	Tumor Type	Time (weeks)
3F	71-678	91-082	Pituitary - Adenoma	104
			Mammary gland - Adenocarcinoma	96
3F	71-679	91-084	Mammary gland - Fibroadenoma	88
3F	71-680	91-087	Pituitary - Adenoma	104
			Thyroid - Carcinoma	104
			Liver - Neoplastic Nodule	104
3F	71-681	91-076	Skin - Keratoacanthoma	88
3F	71-682	91-050	Pituitary - Adenocarcinoma	98
			Uterus - Endometrial Polyp	98
3F	71-683	91-052	Mammary gland - Adenocarcinoma	72
3F	71-685	91-059	Skin - Fibrosarcoma	52
3F	71-687	91-064	Pituitary - Adenoma	99
3F	71-688	91-065	Pituitary - Adenoma	102
3F	71-689	91-067	Adrenal - Cortical Adenoma	101
			Mammary gland - Fibroadenoma	100
3F	71-690	91-068	Mammary gland - Fibroadenoma	76
3F	71-691	91-070	Pituitary - Adenoma	52
3F	71-692	91-071	Pituitary - Adenoma	98
3F	71-693	91-072	Pituitary - Adenoma	101
3F	71-694	91-075	Pituitary - Adenoma	69
3F	71-695	91-077	Pituitary - Adenoma	96
3F	71-696	91-078	Pituitary - Adenoma	98
3F	71-697	91-081	Pituitary - Adenocarcinoma	103
			Mammary gland - Fibroadenoma	100
3F	71-698	91-083	Pituitary - Adenoma	101
3F	71-699	91-085	Lymphoma	19

APPENDIX V-22

LISTING OF MISSING SECTIONS WITH TUMOR DIAGNOSES

BY EITHER UAREP OR EPL

Group	Path No.	Animal No.	Organ	UAREP	EPL
1M	71-502	90-838	Tissue mass	No section	Sarcoma
1M	71-503	90-839	Salivary gland	No section	Reticulum cell sarcoma
1F	71-660	90-927	Pituitary	Adenoma	No section

APPENDIX V-23
NUMBERS OF MALE AND FEMALE RATS WITH HISTOLOGICALLY PROVEN TUMORS
AS DIAGNOSED BY UAREP (U) AND EPL (E)

	Male Groups					
	1		2		3	
	U	E	U	E	U	E
Any tumor	36	32	19	19	20	18
All malignant tumors	15	12	6	4	7	8
Benign tumors	25	20	13	15	16	10
Adrenal cortical tumors	2	-	0	-	1	-
Adrenal medullary tumors	5	-	4	-	1	-
Pituitary tumors	13	-	11	-	8	-
All mammary tumors	0	0	1	1	3	1
Malignant mammary tumors	0	0	0	0	0	0

	Female Groups					
	U	E	U	E	U	E
Any tumor	41	41	31	29	36	34
All malignant tumors	8	11	8	8	11	13
Benign tumors	36	30	27	21	31	21
Adrenal cortical tumors	1	-	2	-	2	-
Adrenal medullary tumors	0	-	2	-	0	-
Pituitary tumors	28	-	18	-	23	-
All mammary tumors	22	18	17	15	12	12
Malignant mammary tumors	2	4	4	3	3	3

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(cont'd) page 2

EPL figures are taken from Figure No. 7 p 54, E-70 which did not contain data on Adrenal or Pituitary tumors. UAREP figures are based on tumors listed in Appendix V-20 and V-21. UAREP'S figures for benign tumors include all rats with diagnoses of benign tumors whereas HLA deleted any rats with benign tumors if they also contained a malignant tumor.

APPENDIX V-24

COMPARISON OF COMPUTATIONS BY UAREP AND HLA OF PROBABILITIES OF
TUMOR INCIDENCE IN MALE AND FEMALE RATS RECEIVING ASPARTAME
OR SERVING AS CONTROLS

Group	Males				Females			
	HLA P	UAREP P	HLA [N]	UAREP [N]	HLA P	UAREP P	HLA [N]	UAREP [N]
Any Tumor								
1	82.4	88.4	38.8	39.6	84.4	89.6	48.6	45.7
2	74.9	83.2	25.4	22.8	87.6	95.0	33.1	32.6
3	63.4	77.3	28.4	25.9	91.6	1.02	37.1	35.3
Benign Tumors								
1	65.5	76.2	30.5	32.8	70.5	86.9	42.6	41.4
2	64.5	67.8	23.3	19.2	75.7	92.3	27.7	29.2
3	37.6*	73.0	26.6	23.3	65.4	95.1	32.1	32.6
Any Malignant Tumors								
1	32.6	55.3	36.8	27.1	25.6	22.9	43.0	34.9
2	15.2	36.3	26.3	16.5	29.7	38.1	26.9	21.0
3	31.5	36.5	25.4	19.2	44.5	46.7	29.2	23.6
All Mammary Tumors								
1	0	6.7	0	14.9	47.0	60.6	38.3	36.3
2	4.7	4.0	21.3	25.0	54.7	63.1	27.4	26.9
3	4.5	18.2	22.2	16.5	39.1	38.5	30.7	31.2
Malignant Mammary Tumors								
1	0	6.7	0	14.9	11.1	8.4	36.0	23.8
2	0	0	0	0	11.2	21.8	26.8	18.3
3	0	0	0	0	9.0	9.3	33.3	32.2

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(cont'd) page 2

Group	Males				Females			
	HLA P	UAREP P	HLA [N]	UAREP [N]	HLA P	UAREP P	HLA [N]	UAREP [N]
Adrenal Cortex								
1		8.9		22.5		5.9		17.0
2		0		0		17.4		11.5
3		8.0		12.5		14.8		13.5
Adrenal Medulla								
1		29.4		17.0		0		0
2		32.0		12.5		12.7		15.7
3		8.0		12.5		0		0
Pituitary Tumors								
1		57.5		22.6		78.4		35.7
2		60.2		18.3		76.2		23.6
3		47.0		17.0		82.6		27.8

HLA data is from Figure No. 8, page 55 of E-70; UAREP data is based on Appendix V-23

HLA did not analyze for tumors of the Adrenal or Pituitary

P=calculated probability, X100, of developing a tumor during the total test period

[N]=estimate of "effective number" of animals on test over the entire period which is number of tumor bearing rats/P

*HLA reported decreased tumor incidence 1>3 at P<0.05; UAREP found no significant differences between groups

APPENDIX V-25
SIGNIFICANT DISCREPANCIES BETWEEN HISTOPATHOLOGIC DIAGNOSIS
BY UAREP AND EPL ON E-70

Group Set	Animal Number	Path Number	Organ	EPL Diagnosis	UAREP Diagnosis
1M	90-812	71-462	Pituitary	Adenoma	Adenocarcinoma
1M	90-815	71-488	Adrenal	X	Cortical adenoma
1M	90-819	71-466	Pituitary	Cyst	Adenoma
1M	90-821	71-492	Skin	0	Lipoma
1M	90-820	71-498	Liver	Hyperplastic nodule	Neoplastic nodule *
1M	90-832	71-470	Mammary gland Skin	0 0	Adenocarcinoma Fibroma, dermatitis 4
1M	90-834	71-471	Thyroid	Adenoma	C-cell carcinoma
1M	90-040	71-473	Pituitary Liver	Adenoma 0	Adenocarcinoma Bile duct hyperplasia and pericholangitis 3
1M	90-841	71-504	Thyroid	0	Parathyroid adenoma
1M	90-845	71-475	Pituitary	Cyst	Adenoma
1M	90-849	71-434	Thyroid	Thyroid adenoma	Parathyroid adenoma
1M	90-853	71-476	Pituitary	Adenoma	Adenocarcinoma
1M	90-857	71-516	Liver	Hepatoma	0
1M	90-858	71-517	Liver	0	Hepatocellular carcinoma
1M	90-860	71-518	Skin	Basal cell carcinoma	X
1M	90-863	71-479	Liver	Hyperplastic nodule	0
1M	90-867	71-402	Pituitary Adrenal	Adenoma 0	Hyperplasia 2 Cortical Adenoma
1F	90-868	71-601	Pancreas Lymph node	Adenocarcinoma Lymphoma	X Lymphoid hyperplasia 4
1F	90-871	71-632	Pituitary Mammary gland	Adenoma 0	Hyperplasia 2 Adenoma
1F	90-873	71-633	Pituitary	0	Adenoma
1F	90-875	71-603	Urinary bladder	Cystitis 2	Fibrous polyp
1F	90-877	71-636	Thyroid Mammary gland Urinary bladder	Parathyroid - adenoma 0 0	C-cell carcinoma Fibroadenoma Papillary epithelial hyperplasia 4
1F	90-879	71-638	Lung	Edema 3	Acute inflammation 3
1F	90-881	71-604	Pituitary	X	Hyperplasia 3
1F	90-886	71-643	Mammary gland	0	Fibroadenoma
1F	90-807	71-606	Mammary gland	Adenoma	Adenocarcinoma

X - indicates section was unremarkable
0 - indicates that no comparable diagnosis was recorded
1-5 degrees of severity of diagnosis as follows:
1-minimal
2-slight
3-moderate
4-moderately severe/high
5-severe/high

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continued, page 2

Group Set	Animal Number	Path Number	Organ	EPL Diagnosis	UAREP Diagnosis
1F	90-888	71-607	Mammary gland	Adenocarcinoma	Adenoma
1F	90-895	71-610	Pituitary	X	Hyperplasia 3
1F	90-898	71-648	Pancreas	X	Anaplastic tumor
1F	90-900	71-613	Liver	Bile duct proliferation 4 fibrosis 4	Bile duct proliferation 1
1F	90-901	71-614	Kidney	Pelvic epithelial hyper- plasia 3	0
1F	90-912	71-654	Pituitary	X	Hyperplasia 4
1F	90-917	71-623	Mammary gland	Adenocarcinoma	0
1F	90-918	71-657	Kidney	Pelvic epithelial hyper- plasia 3	0
			Eye	X	Inflammation 3
1F	90-919	71-658	Liver	0	Neoplastic nodule
1F	90-921	71-625	Adrenal Lymph node	Pheochromocytoma 0	Cortical adenoma Lymphoid hyperplasia 4
1F	90-926	71-629	Mammary gland	0	Fibroadenoma
1F	90-927	71-660	Lymph node Mammary gland Brain	Metastatic tumor 0 Metastatic carcinoma	0 Fibroadenoma - 0
2M	90-930	71-561	Thyroid Testis	Parathyroid adenoma 0	C-cell carcinoma Mesothelioma
2M	90-933	71-563	Prostate	Squamous cell carcinoma	Squamous metaplasia 3
2M	90-934	71-564	Adrenal	0 Pheochromocytoma	Cortical nodular hyperplasia 3 Medullary hyperplasia 5
2M	90-937	71-567	Thyroid	0	Parathyroid adenoma
2M	90-939	71-585	Liver Kidney	Hyperplastic nodule 0	0 Interstitial nephritis and pyelitis 3
2M	90-946	71-589	Large intestine	X	Ulceration with lymphoid hyper- plasia 4
2M	90-947	71-571	Pituitary	X	Adenocarcinoma
2M	90-951	71-573	Lymph node	Reticuloendothelial cell proliferation 4	Lymphoma
2M	90-954	71-574	Thyroid	0	Parathyroid hyperplasia 3
2M	90-955	71-575	Pituitary	Adenoma	Hyperplasia 4
2M	90-956	71-594	Adrenal	0	Pheochromocytoma
2M	90-958	71-595	Kidney	0	Abscess 3
2M	90-959	71-596	Brain	Abscess	X
2M	90-960	71-577	Adrenal	Pheochromocytoma	Medullary hyperplasia 5
2M	90-965	71-580	Thyroid	0	C-cell hyperplasia 5
2F	90-969	71-701	Liver	Hyperplastic nodule	Neoplastic nodule*

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continued, page 3

Group Set	Animal Number	Path Number	Organ	EPL Diagnosis	IJAREP Diagnosis
2F	90-970	71-717	Pituitary Mammary gland	Hyperplasia 3 0	Adenoma Fibroadenoma
2F	90-971	71-702	Thyroid	0	Parathyroid adenoma
2F	90-972	71-703	Adrenal Mammary gland	0 Adenoma	Cortical hyperplasia 4 Adenocarcinoma
2F	90-973	71-704	Adrenal	0	Modular hyperplasia 3
2F	90-974	71-718	Liver	Hyperplastic nodule	Neoplastic nodule *
2F	90-978	71-721	Thyroid	0	Parathyroid adenoma
2F	90-986	71-728	Pituitary	0	Hyperplasia 3
2F	90-987	71-707	Liver	Hyperplastic nodule	Neoplastic nodule *
2F	90-988	71-729	Skin (tissue mass)	Squamous cell carcinoma	Keratoacanthoma
2F	90-992	71-709	Adrenal	0	Cortical adenoma
2F	90-993	71-710	Adrenal Liver	Pheochromocytoma Hyperplastic nodule	Medullary hyperplasia 3 Neoplastic nodule *
2F	90-996	71-733	Adrenal	X	Cortical hyperplasia 3
2F	91-001	71-737	Lung	Interstitial pneumonitis 3	Neoplastic cells in interstitium
2F	91-003	71-714	Liver	Hyperplastic nodule	Neoplastic nodule *
2F	91-007	71-739	Liver Mammary gland Uterus	0 0 0	Basophilic area 4 Papilloma Fibropapilloma
3M	91-015	71-544	Salivary gland	Adenocarcinoma	Hyperplasia 3
3M	91-017	71-527	Pituitary	Cyst	Adenoma
3M	91-018	71-546	Liver	Hyperplastic nodule	0
3M	91-022	71-529	Liver	Hyperplastic nodule	0
3M	91-025	71-550	Skin	0	Keratoacanthoma
3M	91-032	71-536	Liver	Hyperplastic nodule	Neoplastic nodule *
3M	91-035	71-537	Adrenal	Pheochromocytoma	Medullary hyperplasia 2
3M	91-036	71-538	Lymph node	Lymphosarcoma	Lymphoid hyperplasia 5
3M	91-037	71-554	Adrenal Thyroid	Pheochromocytoma Parathyroid adenoma	Medullary hyperplasia 3 C-cell carcinoma
3M	91-043	71-540	Liver	Hyperplastic nodule	0
3M	91-047	71-542	Adrenal	Pheochromocytoma	Medullary hyperplasia 3
3F	91-048	71-661	Liver	0	Eosinophilic area
3F	91-049	71-662	Adrenal Liver	0 Hyperplastic nodule	Modular hyperplasia 3 Neoplastic nodule *
3F	91-050	71-682	Pituitary Uterus	Adenoma 0	Adenocarcinoma Endometrial polyp
3F	91-053	71-664	Adrenal Liver	0 Hyperplastic nodule	Modular hyperplasia 3 Neoplastic nodule *

Appendix V-25
continued, page 4

Group Set	Animal Number	Path Number	Organ	EPL Diagnosis	UAREP Diagnosis
3F	91-054	71-665	Adrenal Spleen Bladder	Pheochromocytoma Reticulum cell sarcoma Metastatic carcinoma	Medullary hyperplasia 2 0 X
3F	91-055	71-666	Pituitary	X	Adenoma
3F	91-056	71-667	Pituitary	0	Hyperplasia 3
3F	91-060	71-669	Pituitary Brain	Adenoma 0	Adenocarcinoma Pituitary tumor infiltrating base of brain
3F	91-061	71-686	Brain	Meningioma	X
3F	91-062	71-670	Pituitary	Hyperplasia 1	Hyperplasia 4
3F	91-063	71-671	Liver Ovary	0 Neurofibrosarcoma	Eosinophilic area 3 Granulosa cell tumor
3F	91-066	71-672	Thyroid Pituitary	Parathyroid adenoma Hyperplasia 2	C-cell carcinoma Adenoma
3F	91-067	71-689	Adrenal Thyroid	Pheochromocytoma 0	Cortical adenoma Parathyroid hyperplasia 3
3F	91-069	71-673	Uterus	Squamous cell carcinoma	Squamous metaplasia 4
3F	91-074	71-675	Liver	Hyperplastic nodule	Neoplastic nodule *
3F	91-076	71-681	Skin (tissue mass)	Squamous cell carcinoma	Keratoacanthoma
3F	91-077	71-695	Pituitary	0	Adenoma
3F	91-079	71-676	Liver	0	Neoplastic nodule
3F	91-083	71-698	Adrenal	0	Nodular cortical hyperplasia 4
3F	91-085	71-699	Brain	X	Acute meningitis 3
3F	91-086	71-700	Adrenal	0	Nodular cortical hyperplasia 3
3F	91-087	71-680	Liver	Hyperplastic nodule	Neoplastic nodule *

*See text in Chapter IV

CHAPTER VI

E75; 104 WEEK ORAL TOXICITY STUDY OF ASPARTAME IN THE MOUSE

INTRODUCTION

Searle Laboratories contracted with Hazleton Laboratories America (HLA) to evaluate and characterize the toxicity during long term dietary feedings of aspartame (Searle Path-Tox Number P-T 984H73; Hazleton 700-259) to weanling mice. Albino mice obtained from Charles River Laboratories, Wilmington, Massachusetts, were used as test animals. The experiments began November 24, 1971 and survivors were sacrificed beginning November 14, 1973. This is a companion experiment to the E-76 studies (Hazleton Project No. 700-260) reported in Chapter VII, with the principal difference being that aspartame was fed instead of diketopiperazine (DKP). The mice were divided into the control, low, medium, and high dose groups. Data were collected on clinical observation, body weight changes, food and compound consumption, survival, ophthalmoscopic observations, hematologic and clinical laboratory studies, tumor incidence and necropsy and histopathologic observations. The methods used will be mentioned with the presentation of Results and Discussions.

General Experiment Design

The total of 360 mice were divided into control groups of 72 males and 72 females, and three treatment groups, each of which contained 36 males and 36 females. The aspartame was manufactured by Searle Laboratories and supplied to Hazleton Laboratories in a number of different

lots. According to Searle, these lots contained between 0.8 and 1.2% of DKP, a conversion product of aspartame. For purposes of diet preparation the aspartame was assumed to be 100% pure. The treatment groups received a low (1 g/kg body weight/day), a medium (2 g/kg body weight/day), and a high (4 g/kg body weight/day) dose of aspartame. Searle estimated that this would be the equivalent of 33, 67, and 133 times the expected human consumption.

Personnel

The following personnel from Searle Laboratories, a division of G. D. Searle and Company, were members of the Searle protocol design committee for E-75:

Dr. Dutt.....Biostatistician
Dr. Sanders.....Biological Research Advisor
Dr. Ranney.....Drug Metabolism Representative
Dr. Polk.....Clinical Representative
Dr. McConnell.....Path-Tox Department Advisor

Dr. Rao of Searle Laboratories, Inc., although not a member of the protocol design committee, acted as liaison between Searle Laboratories and Hazleton Laboratories for experiment E-75.

The following personnel from Hazleton Laboratories, Inc., were involved in E-75:

Dr. Reno.....Project Manager
Dr. Kwapien.....Pathologist
S. Horwatt.....Report Writer
M. Elliott.....Supervisor
Dr. Kundzins.....Ophthalmoscopic Examinations

The histopathology was done by Dr. John F. Ferrell and Dr. William M. Busey of Experimental Pathology Laboratories.

Experimental Animals and Conditions

The mice (CD-1 (HaM/ICR Swiss), four weeks of age at the initiation of the experiment, were housed individually and provided with a powdered diet in special feeders and free access to chlorinated water. The experiments began six days after the mice were received at Hazleton Laboratories. The mice were assigned to various treatment groups by a stratified by weight randomized procedure. They were not rerandomized relative to selection of animals for blood collection for clinical laboratory procedures. A brief description of the animal care facilities at Hazleton is included in Chapter II of this report. The reasons for selecting this specific strain of mouse for the experiments in E-75 and E-76 were not discussed in the materials made available to UAREP by Searle or HLA.

General Comments on Protocol and Amendments

Copies of the Hazleton Project Sheets, Searle protocol, amendments and relevant internal memoranda, are reproduced in Appendix VI-1. The only Searle protocol available to UAREP indicates that it had been amended five times. Although the experiment was originally planned for 80 weeks duration, in May of 1973, approximately 80 weeks after the start of the experiment, it was prolonged until the survival rate in either the control males or control females declined to 25%.

Although the Hazleton project sheets did not mention it, the Searle protocol specified that complete quarterly reports relating to the statistical significant changes in hematology, urinalysis, and clinical chemistries, a general statement relating to general observations, physical examinations, and postmortem examinations of the animals that died, were to be sent to Searle. These reports were to be provided the Director of the Path-Tox Department (Dr. McConnell) on the first of January, April, July, and October, with any serious adverse findings to be reported to him immediately. Although UAREP requested copies of these quarterly reports from both Searle and Hazleton, the only reply received was from Hazleton stating that they had sent copies of these reports to Searle.

Protocol Amendments: The protocol and project amendments are listed at the first of Appendix VI-1. Three of the four Hazleton Project Sheets were supported by Searle protocol amendments. Hazleton Project Sheet No. 2 dated February 14, 1972 which deleted all clinical chemistries and urinalysis determinations from E-75 was not supported by Searle protocol amendment. As part of their E-75 project files, Hazleton provided UAREP six internal Hazleton memoranda relating to changes in E-75. Two of these memos were supported by Searle protocol amendments and the other four changes were not supported by Searle protocol amendments or memos. The type of changes made by Hazleton without evidence of supporting written documentation from Searle included: (1) deletion of the weighing of pituitary glands; (2) discontinuing blood and urine collection; and (3) requesting prothrombin time determinations at the 40th week and at subsequent prescribed intervals.

The protocol provided UAREP by Searle was not dated and the amendment number 5 was circled. UAREP assumed that this was the protocol to which Hazleton referred in their Project Sheet No. 1.

Although the Hazleton Project Sheets do not mention the statistical procedures to be employed, Searle protocol amendment number 1, dated May 21, 1973 indicated that the following statistical and computational procedures were to be used in evaluating the data collected from Experiment E-75: A) body weight change; food and drug consumption: group means \pm standard error; appropriate analysis of intergroup variance at each time interval; B) clinical laboratory values: group means \pm standard error; appropriate analysis of intergroup at each interval; C) incidence and onset of neoplasm: mean incidence and appropriate analysis of intergroup variance at termination.

RESULTS AND DISCUSSION

Clinical Observations

Observations on morbidity and mortality were made daily. Although motor and behavioral activity were noted periodically, no special neurological observations were made. The general external observations included digital palpation for protruding tumor masses and noting body orifices and excrement. Such observations were made at the 5th, 10th, and every succeeding ten weeks thereafter. Clinical observations noted at the time of weighing the mice were recorded weekly for weeks 0-4, every two weeks for weeks 5-12, and every four weeks for weeks 13-104. Information on earlier observations was available on computer output tapes. Such information was numerically coded and reported with body weights and food consumption on the INTEC system, a computerized system for storage and retrieval of animal data utilized by Hazleton.

Although the Hazleton Project Sheet No. 1 and the Searle Protocol specified examination of superficial tumor masses at 5, 10, and every 10 weeks thereafter, the actual observations were made at the time of body weight recording, which was every week for the first four weeks and every two weeks for the next eight weeks, and every four weeks from that point to termination of the experiment, E-75. Thus, examination of individual mice for masses was actually performed more frequently than the protocols specified.

The summary of the palpable tissue masses and nodules is given in Table 6-1. Appendix VI-2 provides a summary of the clinical observations relating to E-75 tumors in which masses or nodules were recorded as being present at one designated observation interval and not observed

Table 6-1

Number of Mice With Palpable Nodules, Tissue Masses,
or Swollen Areas on The Body or Legs

<u>Group</u>	Males		Females	
	<u>UAREP</u>	<u>HLA</u>	<u>UAREP</u>	<u>HLA</u>
1	5/72	5/72	2/72	3/72
2	3/36	3/36	0/36	0/36
3	4/37	4/37	0/35	0/35
4	0/37	0/37	0/35	0/35

at one or more subsequent intervals. As mentioned in the discussion of Chapter IV, UAREP does not necessarily regard such variations in reporting of nodules as equivalent to faulty observation and recording. It is possible to have subcutaneous swellings that relate to normal physiologic variations in fluid distribution or to inflammatory processes.

Body Weight Changes

Body weights of the individual mice were determined at the intervals specified in the protocol. The earliest raw data available to UAREP on these body weights consisted of a computer printout. Scanning of these results did not reveal any figures that would obviously appear to be erroneous, since the computer printout became HLA's input for statistical analysis of body weight data. UAREP did not feel that it was worthwhile for it to duplicate all of the analyses carried out by HLA on body weights. Using this data of E-75 to check the accuracy of the computer used by HLA, was not considered profitable expenditure of time by UAREP.

Food Consumption

The mice received Wayne Laboratory Chow ad libitum throughout the experimental period. Food consumption was recorded at the same intervals as body weight and clinical observations. The food consumption records provided UAREP for the validation of E-75 were computer printouts from the INTEC system utilized by Hazleton Laboratories for storing and

processing the food consumption data, body weights, and clinical observations. The frequency of changing food was not specified in the experiment design.

A summary of the cumulative food consumption over long periods was not computed by UAREP, since this was done in other experiments without producing data significantly different than that in the Entry Reports. A statistical analysis by UAREP of food consumption according to the interval, for computing food consumption is shown in Appendix VI-3. Statistically significant differences ($P < 0.05$) were determined by Analysis of Variance followed by Newman-Keuls and Least Significant Differences tests when ANOVA was $P < 0.05$. The many significant differences reflect the short term fluctuations in food consumption. Of the 29 intervals compared, every possible comparison of the four male and female groups was significantly different at one or more intervals. Among male groups, the most frequent significant differences and (times) observed were: $1 > 4$ (16); $1 > 2$ (13); $1 > 3$ (9); $2 < 3$ (9); $2 > 4$ (9); and $3 > 4$ (9). Among female groups, the significant comparisons were: $1 > 3$ (12); $2 > 4$ (12); $1 > 4$ (11); and $2 > 3$ (10). Group 1 males ate significantly more than Group 2, 3, and 4 at 38 comparisons, while Group 4 ate significantly more than the other groups only 12 times. Similarly, for females, Group 1 ate significantly more than Groups 2, 3, and 4 at 27 intervals and Group 4 ate significantly more than other groups at only 7 intervals. This confirmed a general tendency of high consumption of aspartame to reduce the consumption of food.

Compound Administration

Aspartame was added to the basal diet of laboratory chow and mixed in a twin-shell Patterson-Kelley double sleeve blender. The compound concentration was adjusted weekly for 0-4 weeks, every two weeks for weeks 5 through 12 and every 4 weeks for weeks 12 through 100. Searle and Hazleton both said that there were no subsamples taken from various diets for quality control determinations as to the actual level of aspartame in the various diet mixtures prepared. Dr. Reno of Hazleton indicated that the mixing procedure used had been determined to be adequate for other clients but had not been specifically tested for aspartame. This information was the property of other clients and therefore, was not provided to UAREP by Hazleton. Since the data on compound consumption on an earlier experiment, E-33,34 and one run at approximately the same time, E-76 were both determined in detail and an analysis of compound consumption for E-75 was not made by UAREP. The other two analyses as well as a partial analysis of E-70 showed that the variations in compound administration from the planned dosage were very small and would be of no consequence in a long-term experiment such as E-75.

Survival

Entry Book E-75 summarizes the mean survival time on page 22 of Entry Book E-75 for the control and the respective treatment groups evaluated in Experiment E-75. UAREP recomputed mean survival times for the control in each of the treatment groups, based on death dates recorded under Clinical Observations on the Body Weight and Food

Consumption INTEC computer output tapes. A summary and comparison of the UAREP and Hazleton mean survival time is presented in Table 6-2B. Mean survival time in days as reported in Entry Book E-75 was found to be consistently higher than the mean survival time as computed by UAREP.

UAREP is unable to explain these significant differences since we do not understand how Hazleton obtained figures as high as 699 days mean survival in a 100 week computation in which many animals died prior to the terminal interval.

The data for percent survival at week 100 was computed on the basis of life table analysis. Again, UAREP's figures did not agree with Hazleton's and in seven of the eight comparisons they are lower (Table 6-2A). This was another example in which the UAREP and Hazleton life table analysis data agreed at earlier periods, but not at the later and terminal intervals. This is illustrated in Table 2C showing percent survival at selected intervals. UAREP and HLA agreed that there were no statistically significant differences in survival between groups of mice in E-75.

The terminal sacrifice of mice in E-75 began on November 14 and finished on November 31. Possibly because of this range of dates, HLA indicated on page 22 of E-75 that survival data was based on 100 weeks. UAREP changed their mean survival data to the same 100 week basis. The life table analysis by UAREP in Table 6-2, however, was based on the actual time at which each animal was sacrificed.

Table 6-2

Comparison of UAREP and HLA Data (E-75, page 22) for Mean Percent Survival \pm Standard Error, Mean Survival Time in Days and Percent Survival at Selected Intervals

A. % Survival Rate at Week 100

Group	Males		Females	
	HLA % S.E.	UAREP % S.E.	HLA % S.E.	UAREP % S.E.
1	32.5 \pm 5.6	27.3 \pm 6.1	41.7 \pm 5.9	34.9 \pm 6.6
2	27.8 \pm 7.5	23.8 \pm 8.0	38.9 \pm 8.2	35.4 \pm 9.5
3	25.8 \pm 7.4	21.6 \pm 7.9	41.7 \pm 8.3	32.7 \pm 9.2
4	25.0 \pm 7.3	25.3 \pm 8.4	41.7 \pm 8.3	34.0 \pm 9.2
Life Table	N	N	N	N

B. Mean Survival Time in Days

Group	Males		Females	
	HLA	UAREP	HLA	UAREP
1	699	568	691	611
2	698	582	681	616
3	692	613	693	613
4	698	593	679	593
ANOVA	ND	0.54	ND	0.04
LSD	ND	ND	ND	N
Q	ND	ND	ND	N

Table 6-2
cont'd,

C. Percent Survival at Selected Intervals

Males								
<u>Interval</u>	1		2		3		4	
	<u>HLA</u>	<u>UAREP</u>	<u>HLA</u>	<u>UAREP</u>	<u>HLA</u>	<u>UAREP</u>	<u>HLA</u>	<u>UAREP</u>
13	97	97	100	100	100	100	100	100
26	96	97	100	100	100	100	97	97
52	86	89	97	97	97	97	89	97
78	67	69	56	56	75	78	61	68
91	47	49	36	36	47	50	36	41
100	32	27	28	24	26	22	25	25

Females								
<u>Interval</u>	1		2		3		4	
	<u>HLA</u>	<u>UAREP</u>	<u>HLA</u>	<u>UAREP</u>	<u>HLA</u>	<u>UAREP</u>	<u>HLA</u>	<u>UAREP</u>
13	100	100	100	100	100	100	97	97
26	100	100	97	97	97	97	94	94
52	94	94	86	89	92	91	92	91
78	75	76	69	74	75	74	75	74
91	57	58	50	53	61	60	58	57
100	42	35	39	35	42	33	42	34

Clinical Laboratory Studies

The laboratory studies in E-75 differed from those in Chapter IV and V in that only four chemical parameters were assayed at the termination of the experiment, whereas serial observations were made on six mice of each sex at eight intervals for five hematologic parameters. The use of animals for hematologic and clinical chemical determinations is shown in Appendix VI-4. This appendix shows the mice who had hematologic examinations prior to the terminal interval at which time surviving mice had blood drawn for hematologic and/or clinical chemical analyses.

Hematology - Hematocrit, hemoglobin, erythrocyte count, leukocyte count and differential leukocyte count were determined on the various groups for the intervals of 5, 10, 20, 40, 60, 80, and 104 weeks. The Searle protocol and Hazleton Project Sheets agreed with the parameters determined, as summarized in Appendix Table No. 2, pages 9-53 of Entry Book E-75. The only difference noted was based on a Hazleton internal memo dated April 21, 1972 from Dr. Reno (see Appendix VI-1, Item E) which, for unexplained reasons, indicated that the 20 week hematology determinations were to be repeated as soon as possible, which was done at the 23 week interval. The terminal interval was extended from 80 to 104 weeks because, as indicated in Searle protocol amendment No. 3, the duration was extended until the survival rate declined to 25% for either sex of the control groups.

The Hazleton Project Sheet and Searle Protocol both specified that six animals would be sampled in each of the intervals, including the

terminal one. Normally, the first six animals from each of the respective groups were sampled, with animals being replaced as they were removed from the experiment by natural or accidental death or escape (Appendix VI-4).

Appendix Table No. 2, E-75, reported results on as many as 25 individual mice from Group 1 females which does not agree with the specifications of the Searle protocol or Hazleton Project Sheet. Only on one occasion, animal No. 99791, a Group 3 female, was sampled at week 20, not sampled at week 23 and then sampled again at week 40. This was the only inconsistency in sampling of animals noted by UAREP in their review of the hematology data contained in Appendix Table No. 2. At the terminal 104 week interval, a number of additional animals were sampled for hematology and clinical chemistries. Of the available animals, Appendix VI-4 lists those which were not utilized as blood sources for both hematology and clinical chemistries. These were used for one or the other, but not both.

The initial Searle protocol specified that prothrombin time was to be determined at the terminal 80 week interval. There were no Searle protocol amendments which changed this frequency of determination of prothrombin time. The initial Hazleton Project Sheet dated November 17, 1971 agreed with the initial Searle protocol. However, a Hazleton internal memorandum dated June 29, 1972 indicated (1) that the prothrombin was to be determined at the 40th week interval and at subsequent intervals as indicated in Project Sheet No. 1, (2) that the prothrombin time had already been run at the five and ten week intervals and missed at 20 and 23 week intervals (Appendix VI-1, Item G).

The confidence intervals and means for the hematocrit, hemoglobin, erythrocyte count, leukocyte count, and prothrombin time are shown in Appendix VI-5. The confidence intervals are based on the control group data. The fact that there is considerable variability in the data without any dose related significance is indicated by the fact that many of the mean values for treatment groups were outside the 5% confidence interval. This was true for the means of 14 of 42 hematocrits, 11 of 42 hemoglobins, 10 of 42 erythrocyte counts, 10 of 42 white blood counts, and 5 of 24 prothrombin times. UAREP is unable to evaluate the extent to which this is explained by variables such as inherent biologic differences in the animals, methods of collecting, handling, and analyzing the blood for the various parameters, experiment design, or other factors.

A summary of the discrepancies between the UAREP validation of Appendix Table No. 2 of E-75 is contained in Appendix VI-6. There were no transcriptional errors, between the earliest data source available to UAREP as compared to Appendix Table No. 2 of Entry Book E-75, as well as no computational discrepancies in the 112 means and 112 standard deviations reported in E-75. Four of the five inconsequential rounding discrepancies involved standard deviations and none would alter interpretation of the results. On 23 of the 34 Hazleton's positive t-test results, UAREP agreed. There were, however, 11 additional t-tests on which UAREP did not confirm Hazleton results, although the t-test values in nine of the 11 were close to the significant level. As shown in Appendix VI-7, there were also a few additional instances in which

UAREP compared treatment groups with each other, which HLA did not do, and showed statistically significant differences in values.

Clinical Chemistries - As indicated in Appendix VI-1, there were a number of changes in plans relative to clinical chemistries for E-75. A Hazleton Project Sheet dated February 14, 1972 indicated that all clinical chemistries were to be deleted. Searle protocol amendment No. 3 dated August 20, 1973 and Hazleton Project Memo dated August 24, 1973, specified that insulin, serum ornithine, carbamyl transferase and serum electrophoresis were to be determined. The Searle protocol amendment No. 5 dated November 13, 1973 specified that all surviving mice at terminal sacrifice were to have determinations of blood urea nitrogen, serum glutamic pyruvic transaminase, alkaline phosphatase, insulin, and L-phenylalanine with the latter determination being done by Searle Laboratories. All previous instructions regarding clinical laboratory procedures were superseded by this amendment. Thus, serum ornithine carbamyl transferase and serum electrophoresis and serum insulin were deleted from the plans, although the insulin was added back in Amendment No. 5.

A significant number of the animals listed in Appendix Table No. 3 of the Entry Book E-75 as being alive at the termination of the experiment, were indicated to have an insufficient quantity of blood for the determination of BUN. As shown in Table 6-3, as high as two-thirds of the Group 2 males and Group 1 females had an insufficient sample to determine BUN.

Table 6-3

Number of Mice Used for Blood Sources for Terminal Interval
BUN Determination for E-75

<u>Group</u>	<u>No. Recorded in Entry Book E-75</u>	<u>No. Indicated to Have Insufficient Sample for Determination</u>	<u>% In- sufficient</u>
1M	16	4	25%
2M	9	6	66%
3M	9	4	44%
4M	6	2	33%
1F	25	16	64%
2F	10	2	20%
3F	12	2	17%
4F	10	4	40%

The confidence intervals and means for the clinical chemistry determinations at 103 weeks are given in Appendix VI-8. These confidence intervals ($P < 0.05$) are computed on the control values. The mean values for four of the nine determinations of the experimental male groups fell outside of the 5% level indicating substantial variability in results. Because of the variability of results in the control animals, the confidence intervals themselves exhibited extreme, if not ridiculous, levels--as for example, the BUN range of 28.5 to 151.5.

UAREP's statistical analysis of the clinical chemistry data agreed with the results reported in Entry Book E-75, Appendix Table No. 3 in which no significant differences were found between either the treatment groups and the controls or between treatment groups. This wide range of values in the determinations in the various groups, and the small sample of animals could obscure small but significant biologic variation due to treatment.

UAREP's validation of the data in Appendix Table No. 3 of Entry Book E-75 showed discrepancies listed in Appendix VI-9. No transcriptional discrepancies were found by comparing the contents of Appendix Table No. 3 with the earliest data source available to UAREP. In the 24 means and standard deviations that were computed, there were only the four inconsequential rounding discrepancies and one computational discrepancy. Hazleton reported a statistically significant difference in SGPT for Group 3 males as compared with the controls. UAREP confirmed the significance of the t-test but found an Analysis of Variance value for

the groups of .08, which would not indicate significance at $P < 0.05$ by that test.

L-phenylalanine: The initial protocols of Searle and Hazleton both requested determinations of L-phenylalanine at 5, 10, 20, 40, 60, and 80 week intervals. As mentioned earlier, all clinical chemistries were subsequently deleted but serum L-phenylalanine was requested on all surviving animals by the Searle protocol amendment dated November 13, 1973. Of the 112 animals from which blood samples were obtained at the terminal interval for determinations for hematology and clinical chemistry parameters, only 30 were used for L-phenylalanine. This failure to follow protocol specifications could possibly be explained by the inadequate quantity of serum available from mice. No statistically significant differences were noted in the Entry Book E-75, but the number of animals evaluated in the groups was small as indicated by Table 6-4, with only between 10 and 50% of the animals bled being used for L-phenylalanine determinations. In male groups 2, 3, and 4, there were only 1, 2, and 3 mice respectively on which values were available for computation of means.

In validating the 32 phenylalanine values recorded in Appendix Table No. 2 of Entry Book E-75, UAREP found one transcriptional discrepancy. Mouse No. 99801 was entered with no value for L-phenylalanine recorded adjacent to that number. The background data showed a value of 1.52 and this value was used in computing the mean and standard deviations as reported in Appendix Table No. 3 (p 59). No other discrepancies were noted in this data.

The original protocol specified that L-phenylalanine was to be determined at the prescribed intervals for the controls and high dosage groups unless the high dosage groups were found to be positive. The meaning of the latter term under these circumstances was not clarified. The Hazleton Project Sheet No. 1 specified under Item 19, pharmacologic effects, "Evaluation of the following parameters provides evidence for compound absorption: serum phenylalanine levels." The fact that the phenylalanine determination was done only terminally and that HLA reported no statistically significant increase in serum levels of phenylalanine, could raise the question as to whether or not there was significant absorption of the aspartame.

UAREP's analysis of the differences between L-phenylalanine mean values for the male and female mice fed aspartame is also not statistically significantly increased. The number of mice contributing to each mean was small and the variability in values large (Table 6-5). However, if the values for male and female mice were combined to increase the group size, the high dose mice then had a significantly increased value (t-test $P < 0.05$) as compared with the combined male and female control mice.

Urinalysis

Only analysis for phenylketones by dipstick and microscopic observations of sediment were done at termination. UAREP found no discrepancy between the laboratory data and the results as shown in Table No. 4 pp 60-63 of E-75. Four control, two Group 3, one Group 4 females had 15 mg% phenylketones in their urine.

Table 6-4
Animals Sampled And Used For L-phenylalanine Determination
For 104 Week Interval For E-75

<u>Group</u>	<u>No. Mice Bled¹</u>	<u>No. of Samples L-phenylalanine Determination</u>	<u>% of Samples Used</u>
1M	18	9	50
2M	10	1	10
3M	9	2	22
4M	6	3	50
1F	27	3 ²	15
2F	16	3	19
3F	12	6 ³	50
4F	14	4	29

¹Number of animals bled for hematology or clinical chemistry determinations.

²4 mice bled, but one sample lost

³6 samples used in calculating mean but one omitted in preparation of Appendix Table 3, p 59, E-75

Table 6-5

Serum L-phenylalanine Means \pm Standard Deviation (SD), Range of
Values in mg/dl and Number (N) of Mice Sampled

<u>Group</u>	<u>Dosage</u>	<u>Mean \pm SD</u>	<u>Range</u>	<u>N</u>
1M	0	1.63 \pm 0.53	0.70-2.35	9
1F	0	1.57 \pm 0.38	1.25-1.75	3
2M	1gm	2.15 -	-----	1
2F		1.71 \pm 0.27	1.40-1.92	3
3M	2gm	1.32 \pm 0.01	1.31-1.32	2
3F		2.01 \pm 0.63	1.41-2.74	6
4M	4gm	2.20 \pm 0.80	1.49-3.07	3
4F		2.56 \pm 1.27	1.52-4.34	4

Ophthalmoscopic Observations

The initial Hazleton Project Sheet and Searle Protocol both specified that ophthalmoscopic examinations were to be performed at the 0, 20, 40, 60, and 80 (terminal) week intervals. With the exception of the first and last interval, the examinations were carried out precisely at the time indicated. The initial examination was to be carried out as soon as possible after the start of the experiment and was done on the 4th week, January 4, 1972. At this time, five animals were replaced due to abnormalities in the eyes: (99591), male Group 1; 99611, 99619, and 11620, all three female Group 1; and 99887, female Group 4). These same animal numbers were assigned to new mice replacements and no mention of this change at the 4th week was indicated in the Entry Book discussion of ophthalmoscopic examinations.

The UAREP review of Figure No. 5 (page 32) in Entry Book E-75, found no disagreement between the ophthalmoscopic examination summary form and the Entry Book. The only apparent disagreement noted was the fact that there was additional information contained in the Entry Book which was not available on the summary sheet, such as the fact that the summary sheet would indicate cataracts present but would not indicate in which eye or whether they were unilateral or bilateral. This would appear to indicate that additional information was provided the report writers at Hazleton Laboratories which was not in the raw data supplied UAREP.

Necropsy; Gross Diagnosis; Organ and Organ/Body Weight Ratios

Hazleton Protocol Sheet and Searle Protocol specified that necropsies were to be performed on all animals which died during the course of the study and on animals that were terminally sacrificed. Organ weights were recorded at the terminal interval on each mouse and tissues fixed for sectioning as shown in Appendix VI-1-Item A. Specified organs were weighed and organ/body weight ratios were computed. The specified organ/body weight ratios requested in the protocols are summarized in Entry Book E-75, pages 80-84. Appendix VI-10A gives the results of UAREP's validation of Appendix Table No. 7 listing all discrepancies noted in body weight, organ and organ/body weight ratios. A total of 14 inconsequential rounding discrepancies and 15 computational discrepancies are recorded. Some discrepancies listed as computational could be rounding discrepancies and are of minor magnitude. The individual necropsy sheets on which weights were recorded were the source of data used by UAREP in its validation.

As shown in Appendix VI-10B, HLA reported ten significant t-test results in comparing organ and organ to body weight ratios. These ten were confirmed by UAREP and two additional t-tests at $P < 0.05$ were found.

UAREP detected some confusion regarding the plans of terminating the experiment. The original plans for termination at 80 weeks were extended to such time as only 25% of the control animals were surviving. The actual data on survival of animals was determined by HLA at the 100th week, but the actual sacrifice of animals was not carried out until during the 103rd week. The title of the experiment and many tables referred to E-75 as a 104 week experiment. A careful check by

UAREP did not show a significantly lower incidence of deaths in any one group of animals which would result from determining survival to week 100 instead of week 103. The death of animals after 100 weeks (October 24, 1973) in Group 1 to 4 males were 5/54, 1/27, 0/26, and 4/27 respectively. For female Groups 1 to 4, the deaths at the same time interval were 4/45, 2/23, 3/25, and 2/26 respectively.

The dates on which the mice in various groups were sacrificed are shown in Table 6-6. November 14 represented the start of the 103rd week of the experiment. Although equal numbers of animals in each group were certainly not sacrificed on the same days, the one week difference out of 103 weeks in date of sacrifice would not be expected to produce any significant distortion of the results. UAREP does not know if the animals were sacrificed at the 103rd week instead of the 104th week to avoid disruption of the schedule by Thanksgiving holidays.

Histopathologic Findings

The overall approach to the review of the histopathology findings on E-75 was essentially the same as that described previously for E-33,34 and E-70. The general remarks on histopathology review made in Chapter II of this report also apply to this Chapter VI. The UAREP pathologists reviewing the slides in E-75 and E-76 were from Northwestern University whereas those reviewing E-33,34 and E-70 were from the University of Maryland, but the procedures regarding initial and followup review of slides were comparable.

Table 6-6

Dates on Which Varying Numbers of Surviving Male (M) and
Female (F) Mice Were Sacrificed

<u>Date</u>	<u>Group 1</u>		<u>Group 2</u>		<u>Group 3</u>		<u>Group 4</u>		<u>Total</u>
	<u>M</u>	<u>F</u>	<u>M</u>	<u>F</u>	<u>M</u>	<u>F</u>	<u>M</u>	<u>F</u>	
Nov. 14	17	0	0	0	0	0	5	1	23
15	0	12	0	0	0	0	0	0	12
16	0	10	0	0	0	0	0	0	10
19	0	4	4	1	0	0	1	9	19
20	0	0	0	10	9	0	0	0	19
21	0	0	5	0	1	11	0	0	17
Total sacrificed									
Nov. 14-21	17	26	9	11	10	11	6	10	100
Death after									
Nov. 13	1	0	1	1	0	0	1	2	6

Comparison of EPL and UAREP diagnosis

The slides were initially reviewed without knowledge of EPL's findings; but the same necropsy reports were available as utilized by EPL. The followup review on slides in which significant discrepancies were noted was done with access to EPL's reports. As described in E-33,34 and E-70, this gave UAREP's pathologists an advantage over EPL's pathologists, in that they had a check against transcriptional errors as well as an additional opportunity for reconsidering any specific diagnosis. Rarely, this resulted in the elimination of discrepancies, or the changing of diagnoses to conform more to EPL's diagnoses.

Several recurring problems arose with the mouse histopathology that should be clarified. The incidence of amyloid in the old mice used in this experiment was quite high and many organs of many animals received such a diagnosis. Since amyloid tends to involve multiple organs, UAREP felt it was unreasonable to expect the amyloid diagnoses of both EPL and UAREP pathologists to agree totally with regard to severity and all organ sites involved with such amyloid change. Therefore, UAREP did not list any discrepancies involving the diagnosis of amyloid on any specific organ unless no other organ in that animal received such a diagnosis. For example, it was not considered a discrepancy if EPL diagnosed "amyloid" in the liver and UAREP did not, providing that UAREP diagnosed "amyloid" in some other organs in that same animal. Conversely, a diagnosis of "amyloid" in any specific organ made by UAREP and not by EPL was not considered a discrepancy, if EPL had such a diagnosis listed elsewhere for that particular animal. An extension of this, UAREP

chose to compare, and equate to some degree, diagnoses of "amyloid" with diagnoses of "fibrosis" and "atrophy;" so that discrepancies involving these diagnoses are not listed in Appendix VI-17 unless the degree of severity of the diagnoses was significantly different. As specific stains for amyloid, such as Congo Red, were not available to UAREP or EPL pathologists, it was sometimes difficult to differentiate microscopically between the presence of amyloid and fibrosis in an organ. The problem is further complicated by the frequent coexistence of the two entities. Inasmuch as extensive amyloid or fibrosis of an organ will inevitably lead to atrophic changes in the parenchyma of that organ, the additional diagnosis of "atrophy" was not required on any organ with a diagnosis of amyloid and/or fibrosis, unless of course, the severity was markedly different.

Another recurring area of disagreement between EPL and UAREP pathologists relates to retinal degeneration in the eye. EPL occasionally lists a diagnosis of "retinal degeneration" of a moderate degree in Table No. 8 of E-75. UAREP pathologists felt that all retinal changes seen in those animals were artifactual and chose to ignore them. Such discrepancies, therefore, do not appear in Appendix VI-17.

Tumors - A tabulation of tumors by groups and site of origin is given in Appendix VI-11. There was some difficulty regarding the site of origin of angiosarcomas, as EPL at times listed these tumors as occurring in an organ such as liver or spleen, but with a footnote explaining that the primary site was not identified; eg, animal number 99-556, Group 1 male, page 39, Figure 9, E-75. At times a similar tumor is considered to be primary in the organ diagnosed; eg, animal number 99-597, Group 1 male,

page 39, Figure 9, E-75. For purposes of consistency, UAREP considered angiosarcomas to be primary in the organ where identified, and also that multiple vascular tumors may occur in one animal. This accounts for some differences in the listing of total tumors by organs as shown in Appendix VI-11 between UAREP and EPL figures. In addition, UAREP listed endometrial polyps as tumors, whereas EPL did not, thus producing a higher number for total tumors in some groups of female animals. As a simple check as to whether there were marked differences in tumorigenicity, the average number of tumors per animal is computed in Appendix VI-11. Generally, there is good agreement between the UAREP and EPL data and no evidence of a dose relationship relating to the average number of tumors per animal.

A listing of all tumors diagnosed by UAREP and the probable time of contraction is shown in Appendix VI-12. Because the majority of tumors are not superficial and palpable as are mammary tumors, the time of contraction coincides with the date of death of the animal in most instances. In addition, it is difficult to examine mice clinically with great accuracy regarding masses and nodules, especially when internal organs are involved. As elaborated previously in Chapter IV, all lymphoreticular tumors and hematopoietic tumors are classified under the one heading of "lymphoma" and listed without any designated organ as site of origin.

A few slides which contained tumors according to EPL diagnoses were missing when the UAREP review was done and these are listed in Appendix VI-13. Some organs which received a "no section" diagnosis from EPL were seen by UAREP, and those involving tumor diagnoses are also listed.

The background incidence of tumors observed in this out-bred line of mice maintained in a barrier equipped facility (food pasteurized, equipment sterilized, personnel through a shower walk) was found by Percy and Jonas (1) in 1971 to be 50% in mice 20 months of age or older. Their tumors were 17% lymphoreticular, 22% mammary, 48% pulmonary, and 13% of other types. UAREP's distribution of mice with tumors in all groups was 18% lymphoreticular, 0% mammary, 39% pulmonary, and 57% other types. This illustrates the variability one may encounter in tumor incidence in similar outbred mice maintained under different conditions and the necessity of running adequate controls with such experiments.

Statistical Analysis of Tumor Incidence - Hazleton presented the results of their analysis of tumor incidence in Figure No. 10 and 11 on pages 45 and 46 of E-75. UAREP's analysis of tumor incidence is based on their diagnoses of tumors as shown in Appendix VI-11 and VI-12. The results of UAREP's and HLA's analysis of tumor incidence are compared in Appendix IV-14 and 15.

Both UAREP and EPL analyzed tumors according to the following classification: any tumor, benign tumors, malignant tumors, primary lung tumors, lymphoreticular tumors, and vascular tumors. As shown in Appendix VI-14, there is generally good agreement between UAREP and EPL as to the number of tumors in each of these categories. Some of the categories for benign tumors are higher for UAREP than for EPL. In the category of "benign tumors," EPL included mice that had only a benign tumor. If any animal also had a malignant tumor, it was eliminated from the benign tumor category and counted under malignant tumors only. Under the category of "benign tumors," UAREP counted all animals that

had one or more benign tumors but did not eliminate those also having a malignant tumor. In other words, if a mouse had both a benign and malignant tumor, EPL counted it only under "malignant tumors," whereas UAREP counted the mouse under each category.

As stated in earlier chapters, UAREP applied a modified life table analysis technique. E-75 states that Hazleton employed a life table method of analysis which was followed by a t-test. Neither the Entry Book nor the protocols clarify precisely how this was done.

Data for the probability of developing tumors as derived by UAREP and Hazleton is shown in Appendix VI-15. The life table method on which these data are based takes into consideration not only the number of tumors but the time at which the tumors are presumed to begin. Copies of some of the computer tape data that Hazleton derived were made available to UAREP. However, specific information was lacking as to the number of animals with tumors at various time intervals. Both UAREP and HLA statistical analysis tapes showed the cumulative incidence figures and the standard error. There was generally good agreement in the HLA and UAREP data up to the interval of 90 and 100 weeks with poorer agreement in the data in the terminal weeks. The final figures at the termination of the experiment are the important ones that integrate all of the earlier data. These figures are shown in Appendix VI-15, and reflect the fact that the UAREP and HLA values for analysis of tumor incidence probability do not agree closely.

Of more importance is the fact that both UAREP and HLA applied their statistical analysis to indicate any groups in which the tumor incidence probability would be statistically significantly ($P < 0.05$) different than that in other groups. Hazleton compared the controls

with each of the three experimental groups in each category whereas UAREP compared all of the groups with each of the other groups. Hazleton found Group 2 males had significantly lower incidence for "any tumor" category and that both Group 2 and Group 3 males had statistically significantly less primary pulmonary tumors than controls (Appendix IV-15). UAREP found no statistically significant difference at the 5% level, between any of the groups they compared. Thus, for these experiments, there was no statistical or biological evidence that aspartame had tumorigenic properties.

Non-Neoplastic Diagnoses - A comparison between EPL and UAREP relating to the diagnoses of non-neoplastic lesions comprises Appendix VI-16A, B, C, and D. It must be stressed that the table gives no assurance that matching numbers mean that the same animals received that specific diagnosis, or that the severity of the diagnosis was the same; however, fairly good correlation between EPL and UAREP is shown. This substantiates UAREP's impression that major histopathologic discrepancies and problems in this experiment were relatively few.

The number of times a lesion was diagnosed generally shows good correlation between UAREP and EPL. It is not surprising that there are a few instances in which UAREP and EPL pathologists did not chose to make certain diagnoses with similar frequency. For example, UAREP pathologists as compared with EPL pathologists made the diagnosis of fibrosis of the heart in 52% vs 2% of the sections examined; nodular or diffuse hyperplasia of the liver, or focus of change in 40% vs 2%;

congestion and edema of the lungs in 45% vs 0%; lymphoid hyperplasia or reticuloendothelial hyperplasia of the lymph nodes in 28% vs 3%; interstitial cell hyperplasia of the testis in 44% vs 1%; endometrial hyperplasia of the uterus in 39% vs 17% and amyloid of the spleen in 32% vs 18% of the sections examined by UAREP and EPL pathologists, respectively. The diagnosis of amyloid in most organs other than the spleen was made with about equal frequency. The differences in distribution of these diagnoses between controls and various treatment groups, as in the case of other lesions, did not show any preponderant increase in any one group. The diagnosis of retinal degeneration which was made by EPL pathologists in 16 out of 175 eyes examined, was not made by UAREP pathologists, as mentioned previously. Of these 16 diagnoses, 13 occurred in controls.

A listing of all significant discrepancies in histopathologic diagnoses is shown in Appendix VI-17 of this report. Minor differences in degrees of diagnoses are not shown, nor are discrepancies in relatively inconsequential diagnoses. The discrepancies showed no significant increase in any of the groups of experimental mice. Of these discrepancies, according to the definitions in Chapter II, about 25% would be major, of which half would relate to differences between a benign tumor and a proliferative hyperplasia.

Disease Related to Aspartame - UAREP could find no evidence in these experiments that specific lesions occurred more consistently in aspartame treated groups than controls and as such feels that aspartame had no direct effect in production of tumors or nonneoplastic lesions.

Discrepancies in Following Protocol Design and Correlation of Clinical vs Gross Necropsy vs Microscopic Observations

An extensive correlation of clinical, necropsy, and microscopic findings was not performed in this experiment as was done in E-33,34. As the vast majority of tumors occurred in internal locations, relatively few nodules or masses could have been palpated clinically. UAREP thus felt that an attempt to perform such a correlation was unlikely to be worthwhile. Although a meticulous comparison between the necropsy findings and the microscopic diagnoses was not done, UAREP received the impression from working with the data, that overall good correlation was achieved.

Appendix VI-19 was prepared on the basis of reviewing necropsy sheets on the individual mice. The animals indicated as missing had no individual necropsy sheets in material supplied UAREP and were listed at the first of each group of animals as missing as well as being on the list in Appendix VI-1-Item Q. The tissues on Group 1 male No. 99545 are shown as being fixed on the necropsy sheet but were apparently lost without any explanation provided. Eleven of the animals listed as having advanced or very advanced autolysis had tissues fixed, but histopathologic slides were not prepared. This was substantiated by the absence of any assigned histopathology number and absence of any report on such animals in the Detailed Histopathology Incidence Tables reported in E-75.

The protocol and amendments specified that tissues on all organs for the controls and Group 4 should be sectioned for histopathologic diagnosis. One-third of the animals in Group 2 were to be sectioned and two-thirds of the mice in Group 3, in addition to all significant

lesions recognized grossly in other mice. The brains and urinary bladders were to be sectioned according to special instructions in Searle protocol amendment No. 4 (Appendix VI-1-Item M). There were a number of problems in carrying out these instructions specifically. Of the 36 female mice specified in the protocol for Groups 3 and 4, the sex was inaccurately determined in one in each group so that Groups 3 and 4 males had 37 mice, and Groups 3 and 4 females had 35 mice. Seven mice escaped or were otherwise lost from the experiment (see Appendix VI-1, Item Q). It was reported that there was an advanced degree of autolysis in some animals so that they were not utilized for histopathologic study. In fact, as shown in Figure A, page 11 of E-75, the number of mice not available for histopathologic study comprised 10% or more of the mice in six of the eight groups with the highest losses being 27% of Group 4 males and 14% of the low dose females.

After opening the body cavities, animals which showed advanced autolysis were fixed in formaldehyde without dissection of the organs prior to fixation. Although some may question this technique, a number of UAREP investigators have used a similar method in their own experiments. Care is utilized to see that the body cavities are opened in a manner which permits continuing free access of formaldehyde to the internal organs. In such autolyzed animals, a much more satisfactory dissection of organs can be done after at least partial fixation and even with a substantial degree of autolysis one can frequently demonstrate the presence of tumors by this technique.

UAREP pathologists graded autolysis on a scale of 1 to 5 with 1 being slight and 5 advanced to the degree of making diagnosis difficult. Occasionally the tissues of mice sacrificed in E-75 had a slight degree of autolysis. Sixty-four to 95 percent of the mice in various groups that died had one or more slides which showed some degree of autolysis with the mean degree for groups ranging from 1.2 to 3.6 on the scale of 5. Of the mice that died and showed one or more slides with autolysis, 24% of the sections (201 of 885) were graded as 5 indicating that autolysis made some of the tissues difficult to diagnose.

The deviations from the protocol plans for fixation of tissues for histopathologic observations are summarized in Appendix VI-18. Considering the problems which may arise in a study of this kind, in most instances, the protocol plans were followed closely. In many instances, fewer thyroids and pituitaries were examined than specified. This may be explained in part because when such small organs are embedded in paraffin blocks with other tissue, they may not be present in the plane of section used for the other tissues in the block. There may also be some problems in getting the prostate or vagina in the same plane of section with other tissues in the block. At times small mouse organs may also tend to wash off the slide more readily than larger sections of tissue. The gall bladder is another organ which can be readily missed in sectioning mouse livers. It is not quite so easy to explain the shortage of sections of eye in Group I females or of liver and kidney in Group 3 males. Even though it was specified to be sectioned in the protocol, one would expect difficulty in finding the thymus in old mice. UAREP was unable to note any differences in absence of tissues from specific

groups which it felt would produce an evident bias in the histopathologic interpretations.

CONCLUSIONS

This is a long-term study of the potential toxicity of aspartame involving 360 mice of which there were 72 control males and 72 control females with the remaining mice being divided equally between those receiving 1, 2, and 4 gm/kg/day of aspartame in their food. This was initiated as an 80 week experiment which was subsequently rescheduled to 104 weeks. Survival data were determined only to 100 weeks and the sacrifice of animals began at 103 weeks.

UAREP's validation of clinical observations agreed closely with those by Hazleton Laboratories as far as nodules, swellings, and tumor masses were concerned.

In those categories of survival data in which comparisons were made, UAREP's figures were distinctly lower than Hazleton's. However, both UAREP and Hazleton agreed that there were no statistically significant differences in survival between groups of mice.

Five hematology parameters were analyzed on six animals from each of the various groups at seven different intervals during the course of the experiments. For reasons not stated, the hematology determinations at 20 weeks were repeated at 23 weeks. Although they do not follow the initial protocol plans, prothrombin time determinations were made at early and late intervals in the experiments. Determinations of many of the hematology parameters showed substantial variation in results reported between individual animals. The results of UAREP t-test agreed on 23 of 34 comparisons in which Hazleton found significant results at the 5% level. No transcriptional errors or computational

discrepancies were noted in the validation of the hematology data.

The initial plans for sequential analysis of serum constituents were curtailed and determinations at the termination of the experiment were made for BUN, SGPT, alkaline phosphatase, insulin, and L-phenylalanine. Difficulties were encountered in carrying out all of these analyses on the small amount of mouse blood which was obtained at necropsy. Some of the determinations for BUN were based on as few as four animals and there were only one or two animals in some groups for L-phenylalanine determination. There was considerable variability in the results of the analysis for chemical constituents of serum. Hazleton did not demonstrate a significant increase in the L-phenylalanine levels in the serum in any of the mice fed aspartame. By combining the results for males and females, UAREP was able to demonstrate a statistically significant increase in the L-phenylalanine levels in mice receiving the highest dose of aspartame.

UAREP found no discrepancies in their validation of the urinalysis laboratory data.

Due to the escape of a few animals and the presence of advanced autolysis of others, six of the eight groups of animals had 10 to 27% of the mice lost to histopathologic examination. In a few categories of tissues, sections from the number of animals specified in the protocol were not examined histopathologically. Since these animals were not concentrated in any particular groups, UAREP did not feel that this shortage of specimens significantly affected the results of the histopathologic study.

The review of the histopathologic sections by UAREP agreed reasonably well with the initial diagnoses made by Experimental Pathology Laboratory. There was generally good agreement between UAREP and EPL as to the frequency of various lesions including different types of tumors. UAREP found no statistically increased incidence of tumors among the various groups of mice in the categories of any tumor, all malignant tumors, benign tumors, primary pulmonary tumors, vascular tumors, or lymphoreticular tumors. This differed from Hazleton's finding that Group 2 males had a statistically significant lower incidence for "any tumor" and that both Group 2 and Group 3 males had significantly fewer pulmonary tumors than controls.

UAREP agreed with Searle and Hazleton that under the conditions of these experiments, there was no evidence of any disease predominantly in any of the experimental groups.

REFERENCE

1. Percy, D. H. and Jonas, A. M. Incidence of Spontaneous Tumors in CD^(R)-1, HaM/1CR Mice, J. Nat. Cancer Inst. 46:1045, 1971.

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- 6-1 Number of Mice With Palpable Nodules, Tissue Masses, or Swollen Areas on the Body or Legs
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APPENDIX VI-1

HAZLETON PROJECT SHEETS, SEARLE PROTOCOL,
AMENDMENTS AND INTERNAL MEMORANDA FOR E-75

Hazleton (document & date)	Searle (document & date)	Comments
B. Project Sheet No. 1 (11/17/71)	A. Protocol 10/12/71	Initial design and specification set forth for E-75.
C. Internal Memo (12/28/71)	No supporting document	Discontinued the collection of blood and urine pending further consultation with Searle representative.
D. Project Sheet No. 2 (2/14/72)	No supporting document	Deleted all clinical chemistries and urinalysis determinations from E-75.
E. Internal Memo (4/21/72)	No supporting document	20-week hematology determinations to be repeated, which was done at the 23rd week interval.
F. Project Sheet No. 3 (6/5/72)	L. Protocol amendment No. 2 (5/31/73)	Extended the termination date of experiment E-75 until a 25% survival rate was observed in the controls.
G. Internal Memo (6/29/72)	No supporting document	Run pro-time at 40 weeks and at prescribed intervals thereafter.
H. Internal Memo (7/5/72)	No supporting document	Deleted pituitary weights from Experiment E-75.
J. Internal Memo (5/22/73)	I. Protocol amendment No. 1 (5/21/73)	Changed the termination date of Experiment E-75 which was indicated to be terminated when a 40% survival rate was obtained in the control females or males.

Appendix VI-1
continued, page two

Hazleton (document & date)	Searle (document & date)	Comments
K. Internal Memo (5/31/73)	L. Protocol amendment No. 2 (5/31/73)	Modified the duration of Experiment E-75 indicating that when a 25% survival rate was observed in the males and females, the experiment was to be terminated.
O. Project Sheet No. 4 (8/24/73)	M., N. Protocol amendment No. 3 & 4 (8/20/73)	Added serum insulin, ornithine carbamyl transferase, and serum protein electrophoresis with additional information concerning the histopathological evaluation of the brain and urinary bladder.
No supporting document	P. Protocol amendment No. 5 (11/13/73)	Indicated the terminal clinical chemistries, hematology, and urinalysis determinations to be made and specified the post-mortem procedures for the brain.
Q. Internal Memo (5/20/74)	No supporting document	Listing of missing animals for all experimental groups in E-75.

Alphabetical designation before document indicates chronological sequence of items within Appendix VI-1.

Item A

HAZLETON LABORATORIES PROJECT SHEET

851121

PROJECT SHEET NO. <u>1</u>		Path-Tox No. 984H73		PROJECT NO. <u>700-259</u>	
		PROJECT COORDINATOR Reno/Trutter		DATE November 17, 1971	
COMPOUND(S) SC-18862		LOT NO(S). 76050A		RECEIPT DATE 11-4-71	LH-NUMBER(S) 12,237BB
DIVISIONS PARTICIPATING Toxicology		DISTRIBUTION: CENTRAL FILE (2) EACH DIV. PARTICIPATING EACH DIV. DIRECTOR		SPONSOR PROJ. COORD. DATA PROCESSING	
PHYSICAL AND CHEMICAL PROPERTIES					
SPECIAL PRECAUTIONS (INDICATE PERSONNEL HAZARDS, PROTECTIVE INSTRUCTIONS)					
REFERENCE INFORMATION Searle protocol dated 10-12-71.					
PROGRESS REPORTS DUE Quarterly (Project Manager)		FINAL REPT DUE on completion	INITIALS FER/lgm	SIGNATURE (PROJECT COORDINATOR) <i>[Signature]</i>	
EXPERIMENTAL WORK to be performed in Small Animal Toxicology Section					
<u>SC-18862: 80-Week Oral Toxicity Study in the Mouse</u>					
<u>Animal Groups and Dosage Levels</u> - Charles River ICR Swiss mice; four weeks of age at initiation:					
<u>Group No.</u>	<u>No. of Animals</u> male female		<u>Dosage Levels</u> grams/kg		
1 (Control)	72	72	0		
2	36	36	1		
3	36	36	2		
4	36	36	4		
<u>General Observations</u>	180	180			
Morbidity - mortality: Daily					
Body weight - food consumption: Weekly for four weeks, biweekly for the next eight weeks, and every four weeks thereafter.					
Record pertinent observations as necessitated.					

6873

Project Sheet No. 1
Project No. 700-259 (851121)

- 2 -

November 17, 1971

Ophthalmoscopic Examination - At zero (or as soon as practical), 20, 40, 60, and 80 weeks.

Palpation for Tissue Masses - At zero, five, and 10 weeks, and every 10 weeks thereafter

Clinical Laboratory Studies - As per attached Page 3.

Termination - After 80 weeks of treatment.

Postmortem Procedures - For all animals found dead, killed in extremis, or sacrificed by design as per attached Page 4.

Project Sheet No. 1
Project No. 700-259 (851121)
CLINICAL LABORATORY PROCEDURES*

- Page 3 -

PATH-TOX. PROJ. NO. 984H73
November 17, 1971

Specimen collection: individual**
: (/sex/level)

Blood: Unopettes for hematology. Serum for clin. chem. Na₂EDTA for protine.

Urine: 24 hour specimen collected in individual metabolism cages.

16. HEMATOLOGY

Parameter	No./sex/ level	Rx interval (wks)
Hematocrit.....	6	5,10,20,40,60,80
Hemoglobin.....	6	"
Total RBC.....	6	"
Total WBC.....	6	"
Differential.....	6	"
Reticulocyte.....		
Platelets.....		
Coagulation (L-W).	6	80
Pro. time.....		
Activ. PTT.....		
Warren smear.....		
.....		
.....		

17. URINALYSIS

Parameter	No./sex/ level	Rx interval (wks)
Sp. gravity.....	6	5,10,20,40,60,80
Bili-Labstix.....		
pH, Bilirubin, Protein, Sugar, Ketones, Blood.	6	"
Urobilinogen.....		
Microscopic.....	6	"
Phenylketones***	6	"

18. CLINICAL CHEMISTRY****

Parameter	No./sex/ level	Rx interval (wks)	Parameter	No./sex/ level	Rx interval (wks)
BUN.....	6	5,10,20,40,60,80	GPT.....	6	5,10,20,40,60
Uric acid.....			GOT.....	6	"
Glucose.....	6	"	AP.....	6	"
Sodium.....	6	80	BSP.....		
Potassium.....	6	80	Bilirubin.....	6	80
Calcium.....	6	80	OCT.....		
Fibrinogen.....			CPK.....		
Total Serum Protein.....	6	80	Serum Phenylalanine.....	6	5,10,20,40,60
Serum Cholesterol	6	5,10,20,40,60,80			

* Report actual pre-Rx specimen collection(s) as negative number (wks). Clin. lab
workup done preferably on those animals receiving complete postmortem workup.

** Mice used for clinical lab. work should likewise receive postmortem workup as indicated.

*** C&H group only. Do all groups if H group is positive.

**** CC parameters will be prioritised after receiving the information on availability of micr
methods.

PROTOCOL FOR A PRECLINICAL SAFETY STUDY OF SC-18862

Project Sheet No. 1
Project No. 700-259 (851121)

- Page 4 -

PATH-TOX. PROJ. NO. 984H73

November 17, 1971

19) PHARMACOLOGIC EFFECTS Evaluation of the following parameters provides evidence of compound absorption:

Serum phenylalanine levels.

20) POSTMORTEM PROCEDURES

TISSUES	A Wt.	B Fix	C (Micro)			
			L	M	H	C
Stomach		X	12	24	36	72
Small intestine		X	12	24	36	72
Large intestine		X	12	24	36	72
Lung		X	12	24	36	72
Heart	X	X	12	24	36	72
Kidney	X	X	12	24	36	72
Liver	X	X	12	24	36	72
Gall bladder		X	12	24	36	72
Spleen		X	12	24	36	72
Pancreas		X	12	24	36	72
Pituitary	X	X	12	24	36	72
Thyroid	X	X	12	24	36	72
Adrenal	X	X	12	24	36	72
Gonad	X	X	12	24	36	72
Uterus/sem.v.	X	X	12	24	36	72
Vagina/prostate	M	X	12	24	36	72
Mammary gland R.4&5		X	—	—	36	72
Brain: 2 levels		X	—	—	36	72
Spinal cord		X	—	—	36	72
(brachial plexus) Nerve with muscle		X	—	—	36	72
Eye: Right		X	—	—	36	72
Urinary bladder		X	36	36	36	72
Salivary gland(mand.)		X	—	—	36	72
Lymph node (mesent.)		X	—	—	36	72
Thymus		—	—	—	36	72
Bone marrow(femoral)		X	—	—	36	72
Rib junction		X	—	—	36	72
Skin	—	—	—	—	—	—
Unusual lesions		X	36	36	36	72
Usual lesions		X	36	36	36	72

- A — The organs weighed from each animal.
B — The tissues preserved from each animal.
C — Tissues examined microscopically from the indicated no. of animals of each sex.

Additional postmortem procedures:

Item B

FINAL PROTOCOL FOR A PRECLINICAL SAFETY STUDY OF SC- 18862

- COMMERCIAL LAB PROJ. NO. _____ AMENDED (1)(2)(3)(4)(5) PATH-TOX PROJ. NO. 984873
- 1) Protocol finalized 10-12-71 Treatment initiated 11-24-71 Animals terminated _____ Final report _____ es
fi
- 2) Cpd. needed (kg): Total 14.0 First 4 wks. 0.7 Ordered _____ Del'vy 00 3 es
fi
- 3) Study title & objectives: SC-18862: 80 Week Oral Toxicity Study in the Mouse. P.T. No. 98487
An evaluation of safety during chronic administration to weanling mice, to support
marketing as a food additive in the U.S.
- 4) Species, strain, sex, (M,F): Mouse: *Charles River, Tef. Lungs* M, F. Age (wk) at Rx start: 4
- 5) Rx duration (wks): 80 Route & Freq. of admin.: Continuous, oral, ad lib.
- 6) Mode of admin.: Compound admixed w/w in diat.
- 7) Drug-vehicle mixture stability analysis; Rx wks.: (schedule being devised)
- 8) Est. daily human maximal dose & route: 30 MPK (for 27 Kg child) orally in divided doses.
- 9) Dose levels (MPK daily): Control 0; Low 1; Med. 2; High 4
- 10) Multiple of human dose: 0; 33; 67; 133
- 11) No. & sex of animals/level; 72 M; 36 M; 36 M; 36 M
72 F; 36 F; 36 F; 36 F
- 12) Total animals required: 360
- 13) Housing & basal diet: Individual; mouse complete diet; powdered. Chlorinated tap water.
- 14) General observations (frequency; wks)
Morbidity-mortality: Observe daily
Motor & behavioral activity: Periodically as needed.
Body weight: Weekly up to 4 weeks; biweekly for the next 8 weeks, & once every 4 weeks thereafter.
Food consumption & dose adjust.: Concurrent with body weight interval.
Additional observations: Record pertinent observations.
- 15) Physical examination (frequency; wks)
Gen'l external features, incl. body orifices & excrement: 3, 10, & every 10 wks thereafter.
Limited neurological: _____ Detailed neurological: _____
Ophthalmoscopic and/or slit lamp: At 0, 20, 40, 60 and 80 weeks.
Digital palpation for protruding tissue masses: Pre-Rx.; 3, 10 & every 10 wks thereafter.
Body temperature (rectal): _____
Blood pressure and/or ECG: _____

KSR
10/11/71

Revised
10-11-71

Page 2

PROTOCOL FOR A PRECLINICAL SAFETY STUDY OF CC-1000

16-1F
CLINICAL LABORATORY PROCEDURES*

PATH-TOX. PROJ. NO. 984873

60:9

Specimen collection: (individual)**
(____/sex/level)

Blood: Unopettes for hematology. Serum for clin. chem. Na₂EDTA for protime.

Urine: 24 hour specimen collected in individual metabolism cages.

16. HEMATOLOGY

Parameter	No./sex/ level	Rx interval (wks)
Hematocrit.....	6	5,10,20,40,60,80
Hemoglobin.....	6	"
Total RBC.....	6	"
Total WBC.....	6	"
Differential.....	6	"
Reticulocyte.....		
Platelets.....		
Coagulation (L-W).		
Pro. time.....	6	80
Activ. PTT.....		
Barrow smear.....		
.....		
.....		

17. URINALYSIS

Parameter	No./sex/ level	Rx interval (wks)
Sp. gravity.....	6	5,10,20,40,60,80
Bili-Labstix.....		
pH, Bilirubin, Protein, Sugar, Ketones, Blood.	6	"
Urobilinogen.....		
Microscopic.....	6	"
Phenylketones***	6	"

18. CLINICAL CHEMISTRY****

Parameter	No./sex/ level	Rx interval (wks)	Parameter	No./sex/ level	Rx interval (wks)
BUN.....	6	5,10,20,40,60,80	GPT.....	6	5,10,20,40,60,80
Uric acid.....			GOT.....	6	"
Glucose.....	6	"	AP.....	6	"
Sodium.....	6	80	BSP.....		
Potassium.....	6	80	Bilirubin.....	6	80
Calcium.....	6	80	OCT.....		
Fibrinogen.....			CPK.....		
Total			Serum		
Serum Protein.....	6	80	Phenylalanine.....	6	5,10,20,40,60,80
Serum Cholesterol	6	5,10,20,40,60,80			

* Report actual pre-Rx specimen collection(s) as negative number (wks). Clin. lab workup done preferably on those animals receiving complete postmortem workup.

Mice used for clinical lab. work should likewise receive postmortem workup as indicated.

*** C&H group only. Do all groups if H group is positive.

**** CC parameters will be prioritised after receiving the information on availability of micro-methods.

- 19) PHARMACOLOGIC EFFECTS Evaluation of the following parameters provides evidence of compound absorption: 6000

Serum phenylalanine levels.

- 20) POSTMORTEM PROCEDURES

TISSUES	A Wt.	B Fix	C (Micro)			
			L	M	H	C
Stomach		X	12	24	36	72
Small intestine		X	12	24	36	72
Large intestine		X	12	24	36	72
Lung		X	12	24	36	72
Heart	X	X	12	24	36	72
Kidney	X	X	12	24	36	72
Liver	X	X	12	24	36	72
Gall bladder		X	12	24	36	72
Spleen		X	12	24	36	72
Pancreas		X	12	24	36	72
Pituitary	X	X	12	24	36	72
Thyroid	X	X	12	24	36	72
Adrenal	X	X	12	24	36	72
Gonad	X	X	12	24	36	72
Uterus/sem.v.	X	X	12	24	36	72
Vagina/prostate	M	X	12	24	36	72
Mammary gland R.4&5		X	--	--	36	72
Brain; 2 levels		X	--	--	36	72
Spinal cord		X	--	--	36	72
(brachial plexus) Nerve with muscle		X	--	--	36	72
Eye; Right		X	--	--	36	72
Urinary bladder		X	36	36	36	72
Salivary gland(mand.)		X	--	--	36	72
Lymph node (mesent.)		X	--	--	36	72
Thymus		--	--	--	36	72
Bone marrow(femoral)		X	--	--	36	72
Rib junction		X	--	--	36	72
Skin	--	--	--	--	--	--
Unusual lesions		X	36	36	36	72
Usual lesions		X	36	36	36	72

- A — The organs weighed from each animal.
 B — The tissues preserved from each animal.
 C — Tissues examined microscopically from the indicated no. of animals of each sex.

Additional postmortem procedures:

Page 4

PROTOCOL FOR A PRECLINICAL SAFETY STUDY OF SC-18862

PATH-TOX PROJ. NO. 984873

21) STATISTICAL EVALUATION OF DATA: PROCEDURES USED

a) Body wt. change; food & drug consumption:

Group mean \pm S. E.; appropriate analysis of intergroup variance at each time interval.

b) Clinical laboratory values:

Group mean \pm S. E.; appropriate analysis of intergroup at each time interval.

c) Incidence and onset of neoplasms:

Mean incidence and appropriate analysis of intergroup variance at termination.

d) Randomization procedures:

Simple randomization.

22) INTERIM AND FINAL STUDY REPORTS

The sponsor (Director; Path-Tox Dept) requires a brief quarterly report relating statistically significant changes in items 16, 17, and 18 with a general statement on items 14, 15 and 20, by or on the 1st of Jan., April, July, and October; serious adverse findings are to be reported immediately.

Protocol Distribution List

Design Committee Members:

- 1) Dr. Samarra (Biostatistician)
- 2) Dr. F. Saunders (Biol. Res. Adviser)
- 3) Dr. Ranney (Drug Metab. Rep.)
- 4) Dr. Polk (Clinical represent.)
- 5) Dr. Rao (P-T Dept. monitor)
- 6) Dr. McConnell (P-T Dept. adviser)

Technical Staff:

- 1) _____ (Path. Lab)
- 2) _____ (Autopsy Lab)
- 3) _____ (Bio-Anal. Lab)
- 4) _____ (Gen'l Tox. Lab)
- 5) _____ (Hematology Lab)
- 6) _____ (Pathologist)

Item C

TRW
SYSTEMS GROUP

AVOID VERBAL ORDERS

TO: Elleuth / Protocol Bldg. _____ Mail Sta. _____ Date 12-28-71
FROM: F. E. Reus Bldg. _____ Mail Sta. _____ Ext. _____
SUBJECT: Project No. 700-259

Pending further consultation with the sponsor, you may discontinue the blood chemistry and urine collections on this study.

Item D

HAZLETON LABORATORIES PROJECT SHEET

984H72

851121

PROJECT SHEET NO. <u>2</u>		Path-Tox No. 984H73		PROJECT NO. <u>700-259</u>	
			PROJECT COORDINATOR Reno/Trutter	DATE February 14, 1972	
COMPOUND(S) SC-18862			LOT NO(S). 76050A	RECEIPT DATE 11/4/71	LH-NUMBER(S) 12,237BB
DIVISIONS PARTICIPATING Toxicology			DISTRIBUTION: CENTRAL FILE (2) EACH DIV. PARTICIPATING EACH DIV. DIRECTOR		
Sponsor PROJ. COORD. DATA PROCESSING					
PHYSICAL AND CHEMICAL PROPERTIES					
SPECIAL PRECAUTIONS (INDICATE PERSONNEL HAZARDS, PROTECTIVE INSTRUCTIONS)					
REFERENCE INFORMATION Searle protocol dated 10/12/71.					
PROGRESS REPORTS DUE Quarterly (Project Manager)		FINAL REPT DUE on completion	INITIALS FER:lgn	SIGNATURE (PROJ. COORDINATOR) <i>J. E. Reno</i>	
EXPERIMENTAL WORK to be performed in Small Animal Toxicology Section					

SC-18862: 80-Week Oral Toxicity Study in the MouseModified Protocol

Delete clinical chemistry and urine analysis until further advised.

Item E

TRW
SYSTEMS GROUP

AVOID VERBAL ORDERS

TO: R. Minner / T. Miller / Protocol Bldg. _____ Mail Sra. _____ Date 4-21-72
FROM: F. Reno Bldg. _____ Mail Sra. _____ Ext. _____
SUBJECT: 700-259

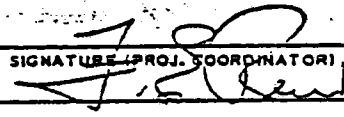
*Please repeat the 20-week karyology determinations for 700-259
ASAP
repeated at 23 WKS*

SYSTEMS PG REV. 9-67

00016

Item F

HAZLETON LABORATORIES PROJECT SHEET

PROJECT SHEET NO. <u>3</u>		P-T No. 984H73		PROJECT NO. <u>700-259</u>	
			PROJECT COORDINATOR	DATE	
			Reno/Trutter	June 5, 1973	
COMPOUND(S)			LOT NO(S)	RECEIPT DATE	LN-NUMBER(S)
SC-18862					12,237
DIVISIONS PARTICIPATING			DISTRIBUTION:		
Toxicology			CENTRAL FILE (2)		
			EACH DIV. PARTICIPATING		
			EACH DIV. DIRECTOR		
			Sponsor		
			PROJ. COORD.		
			DATA PROCESSING		
PHYSICAL AND CHEMICAL PROPERTIES					
SPECIAL PRECAUTIONS (INDICATE PERSONNEL HAZARDS, PROTECTIVE INSTRUCTIONS)					
REFERENCE INFORMATION					
Searle protocol dated 10/12/71					
PROGRESS REPORTS DUE		FINAL REPT DUE	INITIALS	SIGNATURE (PROJ. COORDINATOR)	
Quarterly (Project Manager)		on compl.	FER:da		
EXPERIMENTAL WORK					
to be performed in Small Animal Toxicology					
<u>Amended Protocol</u>					
Duration of this study will be extended. Termination of study will be performed only when the survival rate of either sex in the <u>control group</u> declines to 25% (18/72).					
<p>RECEIVED</p> <p>JUN 6 '1973</p> <p>CHRONIC TOXICOLOGY SECTION</p> <p>00007</p>					

Item G



AVOID VERBAL ORDERS

TO: R25/1 1st/2nd/3rd Bldg. Mail Sta. Date 4-12-72
FROM: R25/1 Bldg. Mail Sta. Ext.
SUBJECT: -252

Policy: In the next clinical interval (Week 40) and at subsequent intervals
as indicated in the form Sheet No. 1, please run prothrombin time determinations,
if possible. This determination was done at Weeks five and 12, but ~~not~~ not
at Week 10 or 11 (no tests).



Item H

- 639 -

HAZLETON LABORATORIES

TO: Minner/Petrovics

CC: Trutter

DATE: July 5, 1972

Technical Data File-

700-247; 700-248;

700-259; 700-260

FILE:

Cottrell

SUBJECT: Pituitary Organ Weights

FROM: Reno *JSK*

BLDG.

ROOM:

Pituitary weights from mice will no longer be required on any 700-studies currently underway. This will preclude any mechanical damage to the organ. However, histological sections will still be prepared as required.

FER:dma

00011

copy P-T file

Item I

607

May 21, 1973

MEMO TO: Sweetener Preclinical Safety Protocol Design Committee Members:

Dr. Dutt (Biostatistician)
Dr. F. Saunders (Biol. Res. Director)
Dr. Ranney (Metab. Rep.)
Dr. Polk (Clin. Rep.)
Dr. McConnell (P-T Dept. Advisor)

COPY TO: Dr. Reno (Hazleton)

From: Dr. Rao

SUBJECT: SC-18862: 80 Week Oral Toxicity Study in the Mouse; P-T 984H73.
Protocol Amendment No. 1.

This eighty week study is due to terminate in June 1973. It was primarily designed to evaluate the tumorigenic potential of SC-18862. This could best be evaluated by exposing the animals to the agent for the maximum duration possible without unduly reducing the survival of animals. For this reason, we decided to extend or continue the study until the mortality of either sex in the control or high dose groups reaches 40%. Study would be terminated at that time. Further modification of this study may arise based on the May 24-25 meeting of NAS-NRC Carcinogenicity Conference.

K. S. Rao

K. S. Rao, Ph. D.

KSR:dv



HAZLETON LABORATORIES

TO:	FILE	CC Johnson	DATE	May 22, 1973
	Project Nos.	✓ Minner	FILE.	
	700-259 & 700-260	Petrovics		
		Trutter		
		Contracts		
		Cottrell		
SUBJECT:	Project Extension		FROM.	Reno
			BLDG.	ROOM

In a telephone conversation with Dr. K.S. Rao from Searle Laboratories, I was advised that the duration of the above referenced projects would be extended. The actual duration is open-ended, with each study to be terminated when the mortality of either sex in the control or high dose group reaches 40%.

/et



HAZLETON LABORATORIES

TO: Minner/Petrovics

CC: Johnson
Trutter
Cottrell

DATE: May 31, 1973

FILE:

SUBJECT: Final Decision, Termination of
Project Nos. 700-259 and 700-260

FROM: Reno

BLDG.

ROOM:

All previous instructions regarding the termination of these studies are hereby cancelled.

Per instructions from Dr. McConnell these studies are to be terminated only when the survival of either sex in the control group reaches 25% (18/72).

Present data indicates that at Week 79 the survival in the control groups are averaging 70%. I would therefore venture that the studies will be extended approximately two months beyond their intended duration.

/et

00013

Item L

May 31, 1973

MEMO TO: Sweetener Preclinical Safety Protocol Design Committee Members

Dr. Dutt (Biostatistician)
Dr. F. Saunders (Biol. Res. Director)
Dr. Ranney (Metab. Rep.)
Dr. Polk (Clin. Rep.)
Dr. McConnell (P-T Dept. Advisor)

COPY TO: Dr. Reno (Hazleton)

FROM: Dr. Rao

SUBJECT: SC-18862: 80 Week Oral Toxicity Study in the Mouse; P-T 984H73.
Protocol Amendment No. 2.

Based on the information acquired at the NAS-NRC Carcinogenicity Conference (May 1973, New York), it was decided to continue the above study until the survival rate declines to 25% in either sex in the control group. In terms of number of animals, this means 18 control mice or more per sex.

K. S. Rao

K. S. Rao

KSR:lg

Ranney
5-31-73

Ref. File

Item M

August 20, 1973

8.0

MEMO TO: Sweetener Preclinical Safety Protocol Design Committee Members:

- 1) Dr. Dutt (Biostatistician)
- 2) Dr. F. Saunders (Biol. Res. Advisor)
- 3) Dr. Ranney (Drug Metab. Rep.)
- 4) Dr. Polk (Clinical Rep.)
- 5) Dr. McConnell (P-T Dept. Advisor)

COPY TO: Dr. Reno (Hazleton Lab.)

FROM: Dr. Rao

SUBJECT: SC-18862: Oral Tumorigenic Study in the Mouse; P-T 984H73.

Protocol Amendment No. 4. Histopathology.

Brain and urinary bladder will be examined from each animal on the study.

Brain: In order to perform a thorough histopathologic evaluation of brain to detect intracranial microscopic tumors, eight coronal slices, 2-4 mm thickness, will be examined grossly and embedded. These slices will be numbered 1 thru 8 from craniad to caudad, and one section of each slice will be examined microscopically.

A schematic representation of any neural tumor and identification of the block, animal and path no. will be included in the report.

Tumor data from the brain will be evaluated in two ways:

- a) taking into consideration all the 8 sections from each animal;
- b) eliminating the data from sections 1 and 8. In other words, use the data from sections 2, 3, 4, 5, 6 and 7 from each animal.

The objective of the latter procedure is to assess the accuracy of evaluating brain tissue from 6 vs. 8 sections, for implementation in future tumorigenic studies.

Urinary Bladder: At necropsy urinary bladder will be slightly distended by injecting neutral buffered formalin into the lumen through the wall. Fixed urinary bladder will be halved longitudinally, examined grossly, both hemispheres embedded, and two longitudinal sections cut from each hemisphere with approximately 50 microns between each section. Hence, four transverse sections from each urinary bladder would be examined microscopically.

K. S. Rao

K. S. Rao

KSR:ja

Revised 8.20.73

Item N

Rept. File

80-3

August 20, 1973

MEMO TO: Sweetener Preclinical Safety Protocol Design Committee Members:

- 1) Dr. Dutt (Biostatistician)
- 2) Dr. F. Saunders (Biol. Res. Advisor)
- 3) Dr. Ranney (Drug Metab. Rep.)
- 4) Dr. Polk (Clinical Rep.)
- 5) Dr. McConnell (P-T Dept. Advisor)

COPY TO: Dr. F. Reno (Hazleton Lab)

FROM: Dr. Rao

SUBJECT: SC-18862: Oral Tumorigenic Study in the Mouse; P-T 984H73.
Protocol Amendment No. 3. Clinical laboratory measurements.

Please make the following additions to the clinical chemistry section for terminal bleeding in all groups (6/sex/group).

- 1) Serum Insulin (Radioimmunoassay).
- 2) Serum Ornithine Carbamyl Transferase (low priority).
- 3) Serum Protein Electrophoresis.

K. S. Rao

K. S. Rao

KSR:lg

Re Met
8-20-73

HAZLETON LABORATORIES PROJECT SHEET

PROJECT SHEET NO. <u>4</u>		P-T No. <u>984E73</u>		PROJECT NO. <u>700-259</u>	
			PROJECT COORDINATOR <u>Reno/Trutter</u>		DATE <u>August 24, 1973</u>
COMPOUND(S) <u>SC-18062</u>			LOT NO(S).	RECEIPT DATE	LH-NUMBER(S)
DIVISIONS PARTICIPATING <u>Toxicology</u>			DISTRIBUTION: CENTRAL FILE (2) EACH DIV. PARTICIPATING EACH DIV. DIRECTOR		Sponsor PROJ. COORD. DATA PROCESSING
PHYSICAL AND CHEMICAL PROPERTIES					
<div style="border: 1px solid black; padding: 5px; display: inline-block;"> RECEIVED AUG 27 '1973 </div>					
SPECIAL PRECAUTIONS (INDICATE PERSONNEL HAZARDS, PROTECTIVE INSTRUCTIONS)					
CHRONIC TOXICOLOGY SECTION					
REFERENCE INFORMATION <u>Searle Protocol dated 8-20-73</u>					
PROGRESS REPORTS DUE <u>Quarterly (Project Manager)</u>		FINAL REPT DUE <u>on compl.</u>	INITIALS <u>FER:da</u>	SIGNATURE (PROJ. COORDINATOR) <u>F.E. Reno / J. Trutter</u>	
EXPERIMENTAL WORK to be performed in Small Animal Toxicology					
<u>Protocol Amendment No. 3 - Clinical Laboratory measurements.</u>					
<p>Please make the following additions to the clinical chemistry section for terminal bleeding in all groups (6/sex/group).</p> <ol style="list-style-type: none"> 1) Serum Insulin (Radioimmunoassay). 2) Serum Ornithine Carbamyl Transferase (low priority). 3) Serum Protein Electrophoresis. 					
<u>Protocol Amendment No. 4 - Histopathology.</u>					
<p><u>Brain:</u> In order to perform a thorough histopathologic evaluation of brain to detect intracranial microscopic tumors, eight coronal slices, 2-4 mm thickness, will be examined grossly and embedded. These slices will be numbered 1 thru 8 from cranial to caudal, and one section of each slice will be examined microscopically.</p> <p>A schematic representation of any neural tumor and identification of the block, animal and path no. will be included in the report.</p> <p>Tumor data from the brain will be evaluated in two ways:</p> <ol style="list-style-type: none"> a) taking into consideration all the 8 sections from each animal; b) eliminating the data from sections 1 and 8. In other words, use the data from sections 2, 3, 4, 5, 6 and 7 from each animal. 					
00008					
HL FORM NO. 5					

Project Sheet No. 4
Project No. 700-259
P-T No. 984H73

- 2 -

August 24, 1973

Urinary Bladder: At necropsy urinary bladder will be slightly distended by injecting neutral buffered formalin into the lumen through the wall. Fixed urinary bladder will be halved longitudinally, examined grossly, both hemispheres embedded, and two longitudinal sections cut from each hemisphere with approximately 50 microns between each section. Hence, four transverse sections from each urinary bladder would be examined microscopically.

OCT

E. electrophoresis

Serum Phenyl. t. Searle

Jan. ? Blood except phenylal.

Mon. p.m.

Serum meth - N. Beaudry - Check w/chem
for radioimmunoassay Draw Sep. immediately
Plasma or serum - Freeze
No carcass Heparinize : 3-4

6 an / sep / gp.

6 / sep / gp

take + freeze

Phenylalanine

Follow protocol

00009

13 Nov 73

003

MEMO TO: SC-18862 Preclinical Safety Studies Protocol Design Committee

Dr. Dutt (Biostatistician)
Dr. F. Saunders (Biological Research Advisor)
Dr. Ranney (Drug Metabolism Representative)
Dr. Polk (Clinical Representative)

COPY TO: Dr. Reno; Hazleton Laboratories

FROM: Dr. McConnell/Dr. Rao

SUBJECT: SC-18862: Oral Tumorigenicity Study in the Mouse; P-T No. 984H73.


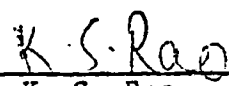
Protocol Amendment No. 5.

- 1) Clinical laboratory measurements. Please make the following measurements on all survivors at terminal sacrifice:
- a. Hematology: hemoglobin, total RBC, total WBC, differential, and hematocrit.
 - b. Urinalysis: microscopic and phenylketones; other conventional measurements if possible.
 - c. Clinical chemistry: GPT, AP, BUN, insulin, L-phenylalanine (Searle Laboratories).

Hazleton Laboratories will ship 0.2 ml of frozen serum to Searle Laboratories for performance of the L-phe measurement; other measurements will be performed at Hazletone Laboratories.

The above supersedes all previous amendments regarding clinical laboratory procedures.

2) Postmortem brain tissue processing. As per Dr. Voelker's memo to Dr. Reno, dated 28 Aug 73, the brain from each animal will be trimmed grossly into 5 transverse blocks; each block will be embedded in a single paraffin block and 6 tissue sections will be prepared. Sections 1 and 6 will be stained and read microscopically; the remaining 4 sections will be saved unstained for future use, as necessary. All information gained from reading slides no. 1 and 6 will be included in the study report. As a separate document, the incidence of brain tumors in slide 1 and in slide 6 will be recorded separately and made available to Searle Laboratories for future reference in planning brain tissue evaluation in mouse tumorigenicity studies. Thus, this SC-18862 mouse study will provide diagnostic information from two tissue sections from each of five transverse blocks of brain from each animal.

 
Dr. R. G. McConnell/Dr. K. S. Rao

RGMcC:ja

Item Q

TO <i>Protocol 700-259</i>		FROM
SUBJECT <i>ANIMALS FOUND MISSING</i>		DATE <i>5-20-74</i>
MESSAGE	<i>Group No. 1 ♂ 99596</i>	{ <i>The preceding animals are those which were found missing during the study.</i>
	<i>Group No. 1 ♀ 99620</i>	
	<i>Group No. 2 ♀ 99716 & 99723</i>	
	<i>Group No. 4 ♂ 99828, 99833, 99846</i>	
SIGNED <i>Jan Trutten</i>		
REPLY TO		DATE
REPLY		
SIGNED		

00017

APPENDIX VI-2

SUMMARY OF VARIATIONS AS REPORTED FOR CLINICAL OBSERVATIONS FOR
SWELLINGS, NODULES AND TISSUE MASSES FOR E-75

<u>Group</u>	<u>Mouse No.</u>	<u>Observations</u>
1M	99545	Firm nodule reported week 68-lower midline
		Not reported week 72
	99547	Swelling reported week 97-right eye
		Not reported week 96
	99549	Swelling reported week 68-lower midline
		Not reported week 72
		Firm nodule reported week 88-right inguinal
		Not reported week 92
		Firm nodule reported week 96-lower midline
		Not reported week 100
	99566	Swelling reported week 44-lower midline
		Not reported week 48
	99588	Protruding anus reported, week 92
		Not reported week 96
1F	99621	Protruding vagina reported week 92
		Not reported week 96

Appendix VI-2
(cont'd) page 2

<u>Group</u>	<u>Mouse No.</u>	<u>Observations</u>
1F	99631	Protruding or swollen vagina reported week 64
		Not reported week 80
		Protruding or swollen vagina reported week 88
		Not reported week 92
		Firm nodule reported week 76-no location
		Firm nodule reported week 80-vagina
		Not reported week 84
		Lower midline reported swollen or bloated, week 84
		Not reported week 96
2M	99688	Firm nodule reported week 96-left inguinal
		Not reported week 100
	99692	Firm nodule reported week 96-back
		Not reported, week 100
	99696	Firm nodule reported week 64-lower midline
		Not reported week 68
3M	99771	Firm nodule reported week 76-left inguinal
		Firm nodule reported week 88-left inguinal
	99781	Not reported week 92
		Firm nodule reported week 84-right inguinal
		Not reported week 92
		Reported week 96
		Not reported week 100

Appendix VI-2
(cont'd) page 3

<u>Group</u>	<u>Mouse No.</u>	<u>Observations</u>
3M	99782	Firm nodule reported week 84-lower midline Not reported week 96
	99784	Swelling reported week 29-right hind paw Not reported week 32
4M	99841	Swollen right eye reported week 80 Not reported week 84
4F	99892	Swollen right hind paw reported week 64 Not reported week 68

APPENDIX VI-3
STATISTICAL COMPARISON OF FOOD CONSUMPTION BY GROUPS
OF MICE AT DIFFERENT INTERVALS

<u>Weeks</u>	<u>Sex</u>	<u>ANOVA</u>	<u>Groups</u>	<u>Q</u>	<u>LSD</u>
1	M	.01	1>2	S	S
			2<3	S	S
	F	.03	1<2	S	S
			2>4	N	S
2	M	.00	1<2	S	S
			1<3	S	S
			2>4	S	S
	F	.00	1<2	S	S
			1<3	S	S
			1<4	S	S
3	M	.00	1>4	S	S
			2>4	S	S
			3>4	S	S
	F	.02	1>3	N	S
			2>3	N	S
			2>4	N	S
4	M	.01	1>2	N	S
			2<3	N	S
			2<4	S	S
	F	.04	1<2	N	S
			2>3	N	S
			2>4	N	S

Appendix VI-3
continued, page 2

<u>Weeks</u>	<u>Sex</u>	<u>ANOVA</u>	<u>Groups</u>	<u>Q</u>	<u>LSD</u>
6	M	.05	1>4	N	S
	F	.00	1>3	S	S
			1>4	N	S
			2>3	S	S
			2>4	S	S
8	M	.00	1>2	S	S
			2<3	S	S
			2<4	S	S
	F	.00	1>2	S	S
			1>4	S	S
			2<3	S	S
			3>4	S	S
10	M	.00	1>2	S	S
			1>3	S	S
			1>4	S	S
	F	.00	1>3	S	S
			1>4	S	S
			2>3	S	S
			2>4	S	S
12	M	.00	1>2	S	S
			1>3	S	S
			2<4	S	S
			3<4	S	S
16	M	.00	1>2	S	S
			1>3	S	S
			1>4	S	S
			2>3	S	S

Appendix VI -3
continued, page 3

<u>Weeks</u>	<u>Sex</u>	<u>ANOVA</u>	<u>Groups</u>	<u>Q</u>	<u>LSD</u>
20	M	.00	1>2	S	S
			1<4	S	S
			2<3	S	S
			2<4	S	S
			3<4	S	S
	F	.00	1<4	N	S
			2<3	S	S
			2<4	S	S
24	M	.00	1>4	S	S
	F	.05	1>3	N	S
29	F	.00	1<2	S	S
			1>4	N	S
			2>3	S	S
			2>4	S	S
32	M	.00	1>2	S	S
			1>3	S	S
			1>4	S	S
	F	.00	1>2	S	S
			1>3	S	S
			1>4	S	S
36	M	.02	1<2	N	S
			2>4	S	S
	F	.00	1<2	S	S
			2>3	S	S
			2>4	S	S
40	M	.00	1>4	S	S
			3>4	N	S
	F	.00	1<2	S	S
			2>3	S	S
			2>4	S	S

Appendix VI-3
continued, page 4

<u>Weeks</u>	<u>Sex</u>	<u>ANOVA</u>	<u>Groups</u>	<u>Q</u>	<u>LSD</u>
44	F	.00	1>3	S	S
			1>4	S	S
			2>3	S	S
			2>4	S	S
48	M	.00	1>2	S	S
			2<3	S	S
			2<4	S	S
			3>4	N	S
52	F	.03	3>4	N	N
	M	.00	1>2	S	S
			1>3	S	S
			1>4	S	S
56	M	.00	1>2	S	S
			1>3	S	S
			1>4	S	S
			2>4	S	S
	F	.00	3>4	S	S
			1>3	S	S
			2>3	S	S
			3<4	S	S
60	M	.00	1>2	S	S
			1>4	S	S
			2<3	S	S
			3>4	S	S
64	M	.03	2<3	N	S
			3>4	N	S
68	M	.00	1>3	S	S
			1>4	S	S
			2>3	S	S
			2>4	S	S
			3<4	S	S

Appendix VI - 3
cont'd, page 5

<u>Weeks</u>	<u>Sex</u>	<u>ANOVA</u>	<u>Groups</u>	<u>Q</u>	<u>LSD</u>
68	F	.00	1>2	S	S
			1>3	S	S
			1>4	S	S
			2>3	S	S
			3<4	S	S
72	M	.00	1>2	S	S
			1>4	S	S
			2<3	N	S
			3>4	S	S
	F	.00	1>3	S	S
			1>4	S	S
			2>4	S	S
76	M	.00	1>4	S	S
			2>4	S	S
			3>4	S	S
	F	.01	1>3	N	S
			1>4	N	S
	M	.00	1>3	S	S
			1>4	S	S
80	F	.00	1>3	S	S
			1>4	S	S
	M	.00	1>3	S	S
			1>4	S	S
	F	.00	1>3	S	S
			1>4	S	S
84	M	.00	1>3	S	S
			1>4	S	S
			2>3	S	S
			2>4	S	S
	F	.00	1>2	S	S
			1>3	S	S
			1>4	S	S
			2>4	S	S
88	M	.00	1<2	S	S
			1>4	S	S
			2>3	S	S
			2>4	S	S
			3>4	S	S

Appendix VI - 3
cont'd, page 6

<u>Weeks</u>	<u>Sex</u>	<u>ANOVA</u>	<u>Groups</u>	<u>Q</u>	<u>LSD</u>
88	F	.00	1>4	S	S
			2>4	S	S
			3>4	S	S
96	M	.00	1<3	S	S
			2<3	S	S
			1<3	S	S
	F	.01	2<3	S	S
			3>4	S	S
			1<4	S	S
100	M	.00	2<4	S	S
			3<4	S	S

ANOVA indicates the exact probability that all group means are equal, based upon the F test for Analysis of Variance. UAREP applied the Analysis of Variance at a difference of $P < 0.05$. When values less than 0.05 were obtained the least significant difference (LSD) and Newman-Keuls (Q) tests were run. If the F statistic was greater than 0.05, we did not do the LSD or Q tests because we accept the hypothesis (at the 5% level) that all the means being compared are equal. All ANOVA values of .00 in this report indicate less than 1% chance that means are equal.

S means $P < 0.05$

N means $P > 0.05$

APPENDIX VI-4

LIST OF MICE IN E-75 USED FOR BLOOD SOURCES FOR HEMATOLOGY (H),
CLINICAL CHEMISTRY (C), AND L-PHENYLALANINE (P).

Male Groups

<u>1</u>	<u>2</u>	<u>3</u>	<u>4</u>
99536 H, C, P	99680 H, C	99752 H	99824 H
99537 H	99681 H	99753 H	99825 H
99538 H	99682 H	99754 H	99826 H
99539 H	99683 H	99755 H	99827 H
99540 H	99684 H, C	99756 H	99828 H
99541 H	99685 H	99757 H, C	99829 H
99545 H	99686 H	99758 H, C	99830 H
99547 C	99687 H, C	99759 H, C	99831 H, C, P
99549 H, C	99688 H, C	99760 H, C	99832 H, C
99552 H, C	99689 H, C	99761 H, C	99833 H, C, P
99553 H, C, P	99690 H, C	99762 H, C	99834 H, C, P
99554 H, C, P	99700 H	99763 H, C	99835 H, C
99555 H, C, P	99701 H, C	99764 H, C, P	99836 H, C
99556 H, C, P	99702 H, C	99765 H, C, P	
99557 H, C, P	99703 H, C, P		
99573 H, C			
99575 H, P			
99576 H, C, P			
99578 H, C			
99583 H, C			
99583 H, C			
99593 H			
99595 H, C			
99601 H, C, P			

Female Groups

<u>1</u>	<u>2</u>	<u>3</u>	<u>4</u>
99603 H	99716 H	99788 H, C, P	99860 H
99609 H	99717 H, C	99789 H	99861 H
99610 H	99718 H	99790 H	99862 H
99611 H	99719 H	99791 H	99863 H, C
99612 H	99720 H	99792 H, C	99864 H
99613 H	99721 H	99793 H	99865 H
99614 H	99722 H, C	99794 H	99866 H
99615 H, C	99726 H, C	99795 H	99867 H, C
99617 H, C, P	99733 H, C, P	99796 H	99870 H, C, P
99619 H, C, P	99737 H, C	99797 H, C, P	99871 H
99621 H	99738 H	99801 H, C, P	99872 H, C, P
99624 H, C	99742 H, C	99802 H, C, P	99881 H, C
99628 H, C	99743 H, C, P	99804 H, C	99882 H, C
99630 H, C, P	99746 H, C, P	99805 H, C	99883 H, C
99631 H, C	99748 H, C	99808 H, C	99887 H, C
99632 H, C	99751 H, C	99809 H, C	99892 H, C, P
99633 H, C		99814 H, C, P	99893 H, C, P
99634 H, C		99818 H, C	
99642 H, C		99823 H, C, P	
99647 H, C			
99647 H, C			
99648 H, C			
99649 H, C			
99650 H, C			
99651 H, C			
99652 H, C			
99655 H, C			
99656 H, C			
99657 C			
99666 H, C			
99670 H, C			
99674 H, C, P			
99675 H, C			
99677 H, C			

H indicates mouse had hematology determination performed.
Those above line were before 104 weeks.

C indicates mouse had clinical chemistry determinations performed
at 104 weeks for BUN, SGPT and alkaline phosphatase.

P indicates blood used for L-phenylalanine determinations
at 104 weeks.

APPENDIX VI-5

CONFIDENCE INTERVALS ($P < 0.05$) AND MEANS FOR Hct, Hgb, RBC, WBC, AND
PRUTHROMBIN TIME FOR E-75

Hematocrit					
Males					
Interval (weeks)	1	2	3	4	Confidence Interval
5	53	51	54	53	50 - 56
10	54	52	53	52	51 - 58
20	39	45*	47*	50*	35 - 43
23	48	48	49	51	45 - 52
40	46	48*	49*	48*	44 - 47
60	48	50	51	52	43 - 53
104	39	45*	45*	37	35 - 44
Females					
5	54	56*	53	53	52 - 55
10	52	56*	54	53	49 - 55
20	43	54	54	53	28 - 58
23	50	50	47*	51	48 - 52
40	45	51*	49	50*	40 - 49
60	46	48	50	46	40 - 53
104	38	42*	41	40	35 - 41
Hemoglobin					
Males					
5	17.6	18.0	18.3	17.8	16.0-19.1
10	16.6	16.1	16.5	16.8	15.1-18.0
20	14.1	15.3	15.8	16.0	11.5-16.7
23	15.6	15.7	16.0	16.0	14.4-16.8
40	15.0	15.1	15.8*	15.7*	14.5-15.5
60	16.2	16.4	16.2	16.0	14.9-17.6
104	12.9	15.4*	15.1*	12.4	11.5-14.3
Females					
5	18.5	19.6*	17.8	18.6	17.8-19.2
10	16.6	17.7*	16.8	16.8	15.8-17.5
20	15.2	15.8	16.6	16.2	13.8-16.7
23	16.2	16.2	15.1*	16.4	15.9-16.6
40	14.1	15.8	16.0*	16.1*	12.3-15.8
60	14.0	15.0	14.9	13.0	11.3-16.8
104	12.5	14.1*	13.8*	13.4	11.6-13.5

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Cont'd page 2

RBC					
Interval (weeks)	Males				Confidence Interval
	1	2	3	4	
5	9.30	9.78	10.22	9.76	8.29-10.31
10	9.06	9.15	9.80	9.02	8.02-10.10
20	8.37	7.41	8.69	8.48	7.37- 9.37
23	8.60	8.38	8.54	8.61	8.12- 9.08
40	9.24	8.70*	8.92	7.77*	8.83- 9.65
60	7.41	7.98	8.16	8.30*	6.63- 8.19
104	7.83	8.52	8.78*	7.10	6.90- 8.76
Females					
5	10.26	10.64	9.64	10.18	9.41-11.13
10	9.34	8.97	9.03	8.91	8.35-10.33
20	7.58	8.14	8.24	8.57*	6.71- 8.45
23	8.12	8.55*	8.69*	9.09*	7.77- 8.49
40	8.82	8.85	8.33	7.86*	8.31- 9.35
60	8.44	9.47	10.11*	9.48	6.86-10.04
104	7.40	7.23	6.79	7.46	6.77- 8.03
WBC					
Males					
5	14.5	11.7	13.5	13.2	11.4-17.6
10	17.4	18.0	13.5*	18.0	13.7-21.1
20	16.4	17.5	13.7	20.9*	12.3-20.5
23	12.3	17.8*	12.2	12.4	8.4-16.3
40	12.4	16.5	14.4	11.9	6.2-18.6
60	16.9	23.7*	19.4	19.7	11.1-22.8
104	28.3	18.8*	26.4	22.2	20.8-35.8
Females					
5	13.4	11.5	10.6	11.1	6.3-20.4
10	14.8	18.4	19.4	14.2	10.2-19.5
20	12.4	14.6	17.0*	15.9*	10.1-14.8
23	11.3	13.2	13.4	11.3	6.6-15.9
40	12.5	9.3	15.5*	17.1*	9.6-15.4
60	16.9	12.3	14.0	12.0	11.0-22.8
104	15.1	14.8	18.8*	15.4	12.8-17.5
Prothrombin Time					
Males					
5	8.35	9.05*	8.95	8.88	7.70-9.00
10	9.22	8.47*	8.72	8.08	8.70-9.74
40	8.43	8.32	8.58	8.47	7.66-9.20
60	8.97	9.62*	9.43	8.75	8.47-9.47
Females					
5	9.03	9.03	8.67	8.32	8.57-9.48
10	8.93	9.30	9.02	7.97*	8.22-9.64
40	8.67	8.12	8.55	7.80*	7.96-9.38
60	8.32	7.77	7.92	8.27	7.27-9.37

*indicates mean values outside confidence interval

APPENDIX VI-6
DISCREPANCIES IN HEMATOLOGY VALUES NOTED IN ENTRY BOOK E-75,
APPENDIX TABLE NO. 2 PAGES 9-53

<u>Interval (week)</u>	<u>Parameter</u>	<u>Group</u>	<u>HLA Value</u>	<u>Type of Discrepancy</u>	<u>UAREP Value</u>
20	Hct	2F	± 2.91	R	2.90 (2.905)
23	Hct	3F	± 2.19	R	2.18 (2.185)
104	Hct	2M	S^+	ST	See Appendix VI-7
104	Hct	2F	S^+	ST	See Appendix VI-7
104	Hct	3M	S^+	ST	See Appendix VI-7
40	Hgb	2F	± 0.61	R	0.60 (0.6058)
104	Hgb	2M	S^+	ST	See Appendix VI-7
104	Hgb	2F	S^+	ST	See Appendix VI-7
104	Hgb	3M	S^+	ST	See Appendix VI-7
5	RBC	1M	9.29	R	9.30 (9.295)
23	RBC	3F	S^+	ST	See Appendix VI-7
40	RBC	2M	S^-	ST	See Appendix VI-7
60	RBC	3M	S^+	ST	See Appendix VI-7
60	RBC	3F	S^+	ST	See Appendix VI-7
60	WBC	4M	± 5.69	R	5.68 (5.6859)
104	WBC	2M	S^-	ST	See Appendix VI-7

Four of these five inconsequential rounding (R) discrepancies involved standard deviations. None would alter interpretation of results. UAREP agreed with HLA on 23 of their positive t-test results. On ten of the 11 above statistical discrepancies UAREP t-tests did not confirm HLA, but nine of the ten t values were close to the significant level.

APPENDIX VI-7
COMPARISON OF UAREP AND HLA STATISTICALLY SIGNIFICANT HEMATOLOGY
GROUP DIFFERENCES

Parameter	Inter- val	Sex	ANOVA	Groups	Q	LSD	UAREP t-test	t-test value	HLA t-test	
Hct	20	M	.01	1<2	S	S	N	(2.19)	S ⁺	
				1<3	S	S	S	2.61	S ⁺	
				1<4	S	S	S	5.66	S ⁺	
	23	F	.01	1>3	S	S	S	2.65	S ⁻	
				2>3	S	S	N	--	ND	
				3<4	S	S	S	3.55	ND	
	40	M	.02	1<2	S	S	N	--	N ⁺	
				1<3	S	S	S	4.51	S ⁺	
				1<4	S	S	S	4.97	S ⁺	
		F	.01	1<2	S	S	S	3.03	S ⁺	
				1<3	S	S	N	--	N ⁺	
				1<4	S	S	S	2.49	S ⁺	
	104	M	.01	1<2	N	N	N	(2.20)	S ⁺	
				1<3	N	N	N	--	S ⁺	
				2>4	N	S	S	3.47	ND	
		F	.16	3>4	N	S	S	3.37	ND	
				1<2	ND	ND	N	--	S ⁺	
	Hgb	5	F	.03	1<2	N	S	N	--	N
					2>3	S	S	S	3.05	ND
23		F	.00	1>3	S	S	S	3.80	S ⁻	
				2>3	S	S	S	3.06	ND	
				3<4	S	S	S	3.41	ND	
40		M	.05	1<3	N	S	S	3.51	S ⁺	
				1<4	N	S	S	3.03	S ⁺	
		F	.01	1<2	S	S	S	2.43	S ⁺	
				1<3	S	S	S	2.81	S ⁺	
				1<4	S	S	S	2.52	S ⁺	
60		F	.14	2>4	ND	ND	S	2.83	ND	
				3>4	ND	ND	S	5.01	ND	
104		M	.01	1<2	N	N	S	2.55	S ⁺	
				1<3	N	N	N	(2.22)	S ⁺	
				2>4	N	S	S	3.44	ND	
		F	.12	3>4	N	S	S	3.16	ND	
				1<2	ND	ND	N	(2.14)	S ⁺	
RBC		5	F	.10	2>3	ND	ND	S	2.65	ND
					RBC				1<4	S
	23	F	.03	1<3	N	N	N	(2.23)	S ⁺	
				1<4	S	S	S	3.05	S ⁺	
	40	M	.00	1>2	N	N	S	2.51	S ⁻	
				1>4	S	S	S	3.31	S ⁻	
				2>4	S	S	N	--	ND	
		F	.01	3>4	S	S	S	2.59	ND	
				1>4	S	S	S	3.02	S ⁻	
	60	M	.29	2>4	S	S	S	2.94	ND	
1<3				ND	ND	N	(2.29)	S ⁺		
	F	.14	1<3	ND	ND	N	(2.21)	S ⁺		

APPENDIX VI-7 (cont.)
page two

Parameter	Inter- val	Sex	ANOVA	Groups	η	LSD	UAREP t-test	t-test value	HLA t-test
RBC	104	M	.08	3>4	ND	ND	S	4.56	ND
WBC	20	M	.08	3<4	ND	ND	S	3.11	ND
	40	F	.04	2<3	M	S	S	2.80	ND
				2<4	S	S	S	2.60	ND
	104	M	.15	1>2	ND	ND	N	--	S ⁻
Pro- time	5	M	.20	1<2	ND	ND	S	2.26	S ⁺
	10	M	.01	1>2	S	S	S	2.58	S ⁻
				1>4	S	S	S	4.42	S ⁻
				3>4	M	S	N	(2.28)	ND
		F	.00	1>4	S	S	S	3.05	S ⁻
				2>4	S	S	S	5.17	ND
				3>4	S	S	S	3.93	ND
	40	F	.29	1>4	ND	ND	S	2.42	S ⁻

ANOVA indicates the exact probability that all group means are equal, based upon the F test for Analysis of Variance. UAREP applied the Analysis of Variance at a difference of $p < 0.05$. When values less than 0.05 were obtained the least significant difference (LSD) and Newman-Kuls (η) tests were run. If the F statistic was greater than 0.05, we did not do the LSD or η tests because we accept the hypothesis (at the 5% level) that all the means being compared are equal.

S means $p < 0.05$.

N means $p > 0.05$.

UAREP used the two tailed t-test with 8 degrees of freedom, $t_{0.05} = 2.306$. HLA has not specified their critical value for $t_{0.05}$. They compared experimental groups with controls whereas UAREP did intergroup analysis comparison of all groups.

ND = not done

APPENDIX VI-8

CONFIDENCE INTERVALS ($P < 0.05$) AND MEANS FOR 104 WEEK CLINICAL
CHEMISTRY PARAMETERS FOR ENTRY BOOK E-75

Male Groups					Confidence Interval
<u>Parameter</u>	<u>1</u>	<u>2</u>	<u>3</u>	<u>4</u>	
BUN(mg%)	55.8	89.7*	61.0	51.0	46.8 -- 64.7
SGPT(R-F)	47.5	45.6	75.1*	47.4	32.6 -- 62.4
Alk. Phos. (K-A)	9.9	25.9*	14.6*	12.3	7.0 -- 12.8

Female Groups					Confidence Interval
<u>Parameter</u>	<u>1</u>	<u>2</u>	<u>3</u>	<u>4</u>	
BUN(mg%)	90.0	50.6	82.6	82.1	28.5 -- 151.5
SGPT(R-F)	45.4	49.9	45.8	55.8*	38.3 -- 52.5
Alk. Phos. (K-A)	14.5	16.2	15.1	13.8	9.7 -- 19.3

* Indicates mean values outside confidence interval

APPENDIX VI-9

DISCREPANCIES NOTED BY UAREP IN BLOOD CHEMISTRY VALUES AT 104 WEEKS
IN APPENDIX TABLE NO. 3, ENTRY BOOK E-75, PAGES 54-58

<u>Parameter</u>	<u>Group</u>	<u>HLA Value</u>	<u>Type of Discrepancy</u>	<u>UAREP Value</u>
SGPT	3M	75.13	R	75.12 (75.125)
BUN	2F	50.63	R	50.62 (50.625)
BUN	3F	±45.09	R	45.08 (45.0856)
SGPT	4F	±31.28	C	31.21 (31.2158)
Alk. Phos.	4F	±5.623	R	5.622 (5.6225)

APPENDIX VI-10A

DISCREPANCIES NOTED IN UAREP REVIEW OF APPENDIX
TABLE NO. 7, PAGES 80-84
OF E-75

<u>Parameter</u>	<u>Group</u>	<u>HLA Value</u>	<u>Type of Discrepancy</u>	<u>UAREP Value</u>
Thyroid Ratio	3M	±.019	R	.018 (.0184)
Thyroid Ratio	1F	.047	C	.045 (.0454)
Thyroid Ratio	1F	±.014	C	.016 (.0165)
Thyroid Weight	4F	.017	R	.016 (.0165)
Thyroid Ratio	4F	.057	R	.058 (.0578)
Heart Ratio	3M	0.70	C	0.69 (.694)
Heart Ratio	3M	±.09	C	0.80 (.081)
Heart Ratio	1F	.66	R	0.67 (0.666)
Heart Ratio	2F	.65	R	0.66 (0.655)
Liver Ratio	2M	±2.94	C	2.89
Liver Weight	3M	1.63	R	1.62 (.1625)
Liver Ratio	3M	5.15	R	5.14 (5.136)
Liver Ratio	3M	±1.03	C	1.00
Liver Ratio	2F	5.73	C	5.76
Liver Ratio	2F	±0.68	R	0.67 (0.670)
Liver Ratio	3F	±1.06	R	1.05 (1.052)
Liver Ratio	4F	5.72	R	5.73 (5.727)

Appendix VI-10A
(cont'd) page 2

<u>Parameter</u>	<u>Group</u>	<u>HLA Value</u>	<u>Type of Discrepancy</u>	<u>UAREP Value</u>
Kidney Ratio	3M	0.4	C	0.3 (.339)
Kidney Weight	4M	0.58	C	0.68
Kidney Weight	4M	0.26	C	0.14
Kidney Ratio	4M	1.9	C	2.25
Kidney Ratio	4M	0.8	C	0.369
Kidney Ratio	3F	0.2	C	0.011
Adrenals Ratio	1M	0.037	R	0.036 (0.363)
Adrenals Ratio	2M	0.047	R	0.048 (0.476)
Adrenals Weight	4F	0.001	R	0.002 (0.0015)
Adrenals Weight	4F	.	ST	See Appendix VI-10B
Testes Weight	2M		ST	See Appendix VI-10B
Testes Weight	3M	0.16	R	0.17 (0.167)
Testes Ratio	3M	0.50	C	0.52
Testes Ratio	3M	0.11	C	0.10 (0.101)

APPENDIX VI-10B

COMPARISON OF UAREP AND HLA STATISTICALLY SIGNIFICANT BODY
WEIGHT AND ORGAN BODY WEIGHT RATIOS

<u>Parameter</u>	<u>Sex</u>	<u>ANOVA</u>	<u>Groups</u>	<u>Q</u>	<u>LSD</u>	<u>UAREP t-test</u>	<u>t-test Value</u>	<u>HLA t-test</u>
Thyroid Weight	M	.004	1<2	S	S	S	4.10	S ⁺
			1<4	N	N	S	2.18	S ⁺
Thyroid Ratio	M	.007	1<2	N	S	S	4.07	S ⁺
Thyroid Weight	F	.001	1<2	S	S	S	4.00	S ⁺
			2>3	S	S	S	2.67	N.D.
Thyroid Ratio	F	.003	1<2	S	S	S	3.62	S ⁺
			2>3	N	S	S	2.86	N.D.
Heart Weight	F	.008	1<4	S	S	S	3.39	S ⁺
			2<4	S	S	S	2.67	N.D.
			3<4	S	S	S	3.44	N.D.
Heart Ratio	F	.006	1<4	S	S	S	3.26	S ⁺
			2<4	S	S	S	2.91	N.D.
			3<4	S	S	S	4.20	N.D.
Adrenal Weight	F	.038	1<4	N	S	S	3.32	N
			2<4	N	S	S	2.82	N.D.
Testes Weight		.040	1>2	N	N	S	2.51	N
Prostate Weight		.004	1>3	N	S	S	3.48	S ⁻
Prostate Ratio		.004	1>3	N	S	S	3.52	S ⁻
			1>2	N	N	S	2.23	S ⁻
			3<4	N	S	S	2.85	N.D.

The footnotes shown on Appendix VI-7 also apply to this table.

APPENDIX VI-11
COMPARISON OF UAREP (U) AND EPL (E) DATA ON FREQUENCIES OF TYPES OF
HISTOLOGICALLY PROVEN TUMORS IN MALE AND FEMALE MICE RECEIVING
ASPARTAME OR SERVING AS CONTROLS

	Male Groups								Female Groups							
	Group 1	Group 1	Group 2	Group 2	Group 3	Group 3	Group 4	Group 4	Group 1	Group 1	Group 2	Group 2	Group 3	Group 3	Group 4	Group 4
	U	E	U	E	U	E	U	E	U	E	U	E	U	E	U	E
Adrenal	55	57	13	13	23	23	26	26	61	63	12	11	21	22	29	29
Cortical adenoma	0	0	0	0	0	0	0	0	0	0	0	0	1	1	0	0
Cortical carcinoma	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Thyroid	37	45	1	1	5	6	7	14	22	29	7	8	17	18	18	22
Adenoma	0	0	0	0	0	0	0	0	1	1	0	0	0	0	0	0
Liver	63	65	17	13	29	29	27	27	65	65	14	14	27	27	30	30
Neoplastic nodule	2	0	1	1	0	0	1	0	2	0	0	0	0	0	0	0
Hepatocellular carcinoma	0	3	0	0	1	0	0	1	1	1	0	0	0	0	0	0
Angioma	1	0	1	0	0	0	0	0	1	1	0	0	0	0	1	1
Angiosarcoma	1	2 ^d	0	0	0	0	0	0	2	2	0	0	0	0	0	0
Bile duct carcinoma	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0
Lung	62	63	23	23	26	26	26	25	65	65	23	23	29	29	29	30
Alveolar adenoma	8	9	1	1	3	3	2	2	6	7	4	3	3	2	4	4
Adenocarcinoma	1	1	0	0	1	0	1	1	2	0	0	1	0	0	0	0
Pancreas	59	62	16	15	23	20	27	27	62	60	14	12	27	25	27	30
Adenoma	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0
Stomach	64	64	19	19	25	25	27	27	64	66	17	15	26	27	28	29
Carcinoma	1	1	0	0	0	1	0	0	0	0	0	0	0	0	0	0
Small Intestine	61	61	15	13	28	21	21	24	62	62	15	14	25	21	28	26
Angiosarcoma	1	1	0	0	0	0	0	0	0	0	0	0	0	0	1	0
Lymph node	46	45	5	2	22	1	23	22	58	52	15	9	18	4	27	23
Angioma	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	1
Spleen	56	57	15	14	24	24	25	25	65	63	15	15	27	27	30	29
Angioma	0	0	0	0	1	0	0	0	2	2	0	0	0	0	0	0
Angiosarcoma	0	0	0	0	0	1	1	1	0	0	0	0	1	1	0	0
Kidney	64	65	21	21	29	29	27	27	65	66	19	19	27	27	30	30
Angiosarcoma	0	0	0	1	0	0	0	0	0	0	0	0	1	0	0	0
Bladder	61	63	29	20	33	33	27	25	59	61	27	28	26	26	22	24
Carcinoma	0	0	0	1	0	0	0	1	0	0	0	0	0	0	0	0
Ovary									57	57	11	11	19	21	25	26
Luteoma					Not applicable				2	0	3	2	1	0	1	1
Vagina									43	44	10	8	21	16	20	23
Carcinoma					Not applicable				0	0	0	1	0	0	0	0

*EPL total differs from UAREP total because UAREP included endometrial polyps as tumors. EPL did not.

^dAnimal number 99-556, Group 1 Male, is listed in Figure No. 9, page 39, E-75 as "Liver-Angiosarcoma" with a footnote indicating the primary site was not determined. UAREP chose to consider the liver as the primary site in this case.

The numbers opposite the organ indicate the total number of animals with sections of that organ examined.

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continued, page 2

	Group 1		Male Groups				Group 4		Female Groups				Group 3		Group 5	
	U	E	Group 2		Group 3		U	E	Group 1		Group 2		U	E	U	E
Uterus									64	63	23	22	28	29	31	31
Endometrial polyp			Not applicable						4	2	1	0	2	1	0	0
Angioma									1	2	1	1	0	0	0	0
Leiomyoma									1	1	1	1	0	0	0	0
Sarcoma									0	1	0	0	0	0	0	0
Eye	59	60	0	0	1	1	26	26	56	56	0	0	1	1	27	30
Lacrimal gland adenoma	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0
Tissue mass/skin																
Angioma	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Angiosarcoma	0	1	0	0	0	0	0	0	0	0	0	0	1	2	0	0
All Organs																
Lymphoma	2	2	0	0	0	0	2	2	7	7	2	3	1	1	4	4
Total number of tumors (UAREP count)	18	21	3	4	6	6	7	8	34	27	12	12	13	8	11	12
Original EPL totals		20		4		6		8		25*		12		7	8	12
Average number of tumors per animal	.25	.3	.1	.1	.2	.2	.2	.2	.5	.4	.4	.4	.4	.2	.3	.3

APPENDIX VI-12

MICE WITH TUMORS HISTOLOGICALLY PROVEN BY UAREP WITH WEEK OF
PRESUMED INITIAL OBSERVATION OF TUMOR

Group	Path No.	Animal No.	Tumor Type	Time (weeks)
1M	100-002	99-547	Lung - Alveolar adenoma	104
1M	100-003	99-549	Lung - Alveolar adenoma	104
1M	100-006	99-559	Lymphoma	104
1M	100-008	99-566	Lung - Alveolar adenoma	104
1M	100-009	99-567	Lung - Alveolar adenoma	104
			Small intestine (tissue mass) - Angiosarcoma	104
1M	100-011	99-575	Lung - Alveolar adenoma	104
			Stomach - Carcinoma	104
1M	100-014	99-583	Lung - Alveolar adenoma	104
1M	100-015	99-588	Lung - Adenocarcinoma	104
1M	100-016	99-595	Liver - Neoplastic nodule	104
1M	100-017	99-601	Lung - Alveolar adenoma	104
1M	100-027	99-546	Lung - Alveolar adenoma	77
1M	100-033	99-556	Liver - Angiosarcoma	79
1M	100-043	99-572	Liver - Neoplastic nodule	96
1M	100-056	99-593	Lymphoma	103
1M	100-058	99-597	Liver - Angioma	87
1M	100-059	99-598	Skin - Angioma	98
2M	100-095	99-680	Lung - Alveolar adenoma	104
2M	100-103	99-706	Liver - Neoplastic nodule	104
2M	100-126	99-715	Liver - Angioma	88
3M	100-129	99-758	Lung - Alveolar adenoma	104
3M	100-133	99-701	Spleen - Angioma	104
3M	100-137	99-754	Lung - Alveolar adenoma	97
3M	100-141	99-760	Lung - Adenocarcinoma	93
3M	100-145	99-768	Lung - Alveolar adenoma	84
3M	100-151	99-752	Liver - Hepatocellular carcinoma	76
4M	100-068	99-838	Liver - Neoplastic nodule	104
4M	100-069	99-842	Spleen - Angiosarcoma	104
4M	100-073	99-839	Lymphoma	103
4M	100-076	99-826	Lymphoma	72
4M	100-090	99-851	Lung - Alveolar adenoma	103
4M	100-093	99-857	Lung - Alveolar adenoma	91
4M	100-321	99-869	Lung - Adenocarcinoma	104
1F	100-162	99-615	Uterus - Endometrial polyp	104
1F	100-163	99-617	Lung - Alveolar adenoma	104
			Uterus - Endometrial polyp	104
1F	100-164	99-619	Uterus - Endometrial polyp	104
1F	100-165	99-621	Spleen - Angioma	104

Appendix VI-12
cont. page 2

Group	Path No.	Animal No.	Tumor Type	Time (weeks)
1F	100-166	99-624	Liver - Angiosarcoma	104
1F	100-167	99-628	Uterus - Endometrial polyp	104
1F	100-171	99-633	Ovary - Luteoma	104
1F	100-172	99-634	Liver - Hepatocellular carcinoma	104
1F	100-173	99-642	Spleen - Angioma	104
1F	100-174	99-647	Lymphoma	104
1E	100-175	99-648	Liver - Neoplastic nodule	104
			Lung - Adenocarcinoma	104
1F	100-179	99-652	Lymphoma	104
1F	100-180	99-655	Uterus - Angioma	104
1F	100-182	99-657	Lung - Alveolar adenoma	104
1F	100-184	99-670	Liver - Neoplastic nodule	104
1F	100-186	99-675	Lung - Alveolar adenoma	104
1F	100-187	99-677	Ovary - Luteoma	104
1F	100-188	99-608	Lymphoma	98
1F	100-189	99-609	Thyroid - Adenoma	65
1F	100-190	99-610	Lung - Alveolar adenoma	98
1F	100-195	99-616	Pancreas - Adenoma	72
1F	100-200	99-626	Lung - Alveolar adenoma	82
			Lung - Adenocarcinoma	82
1F	100-204	99-636	Liver - Angiosarcoma	88
1F	100-208	99-641	Lung - Alveolar adenoma	94
1F	100-210	99-644	Liver - Angioma	65
			Lymphoma	65
1F	100-215	99-659	Lymphoma	62
1F	100-218	99-662	Lymphoma	84
1F	100-222	99-669	Eye - Lacrimal gland adenoma	99
			Uterus - Leiomyoma	99
			Lymphoma	99
2F	100-262	99-726	Ovary - Luteoma	104
2F	100-263	99-733	Ovary - Luteoma	104
2F	100-266	99-743	Lymphoma	104
2F	100-270	99-720	Ovary - Luteoma	102
			Uterus - Angioma	104
2F	100-274	99-724	Uterus - Endometrial polyp	104
2F	100-276	99-728	Lung - Alveolar adenoma	83
2F	100-279	99-731	Lung - Alveolar adenoma	92
2F	100-283	99-738	Lung - Alveolar adenoma	104
2F	100-285	99-740	Lung - Alveolar adenoma	85
			Lymphoma	85
2F	100-289	99-750	Uterus - Leiomyoma	85
3F	100-292	99-798	Uterus - Endometrial polyp	104

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cont. page 3

Group	Path No.	Animal No.	Tumor Type	Time (weeks)
3F	100-294	99-802	Lymph node - Angioma	104
3F	100-300	99-823	Uterus - Endometrial polyp	104
3F	100-302	99-791	Kidney - Angiosarcoma	86
3F	100-303	99-795	Lung - Alveolar adenoma	83
3F	100-305	99-799	Lung - Alveolar adenoma	103
3F	100-306	99-800	Adrenal - Cortical adenoma	98
			Ovary - Luteoma	98
3F	100-307	99-803	Lung - Alveolar adenoma	97
			Spleen - Angiosarcoma	97
3F	100-315	99-793	Mesentery (tissue mass) - Angiosarcoma	59
3F	100-319	99-813	Lymphoma	101
4F	100-229	99-870	Small intestine - Angiosarcoma	104
4F	100-231	99-872	Lymphoma	104
4F	100-235	99-887	Lung - Alveolar adenoma	104
4F	100-237	99-893	Liver - Angioma	104
4F	100-245	99-874	Lymphoma	64
4F	100-246	99-875	Lymphoma	83
4F	100-250	99-884	Lung - Alveolar adenoma	82
4F	100-254	99-889	Lymphoma	102
4F	100-255	99-890	Ovary - Luteoma	76
4F	100-256	99-891	Lung - Alveolar adenoma	88
4F	100-257	99-894	Lung - Alveolar adenoma	104

UNREP recognizes that data on the time of onset of tumors is grossly approximate when using criteria either of (a) time of sacrifice or death with proven tumor not previously recognized, or (b) first date of clinical observation of swelling subsequently confirmed histologically as tumor. Better data is not available

APPENDIX VI-13

LISTING OF MISSING SECTIONS WITH TUMOR DIAGNOSES
BY EITHER UAREP OR EPL

Group	Path No.	Animal No.	Organ	UAREP	EPL
1M	100-033	99-556	Spleen	No section	Angiosarcoma
2M	100-112	99-694	Urinary Bladder	No section	Carcinoma
2F	100-263	99-733	Ovary	Luteoma	No section
4F	100-229	99-870	Small intestine	Angiosarcoma	No section

APPENDIX VI-14

NUMBERS OF MALE AND FEMALE MICE WITH HISTOLOGICALLY PROVEN TUMORS
AS DIAGNOSED BY UAREP (U) AND EPL (E)

	Male Groups							
	1		2		3		4	
	U	E	U	E	U	E	U	E
Any tumor	16	17	3	4	6	6	7	8
All malignant tumors	6	11	0	3	2	3	4	6
Benign tumors	12	6	3	1	4	2	3	3
Primary pulmonary tumors	9	10	1	1	4	3	3	3
Vascular tumors	4	3	1	1	1	1	1	1
Lymphoreticular tumors	2	2	0	0	0	0	0	2

Female Groups								
Any tumor	28	21	10	8	10	7	11	12
All malignant tumors	12	11	2	4	4	4	5	5
Benign tumors	20	10	9	4	7	3	6	7
Primary pulmonary tumors	7	7	4	4	3	2	4	4
Vascular tumors	6	7	1	1	4	3	2	2
Lymphoreticular tumors	7	7	2	3	1	1	4	4

EPL data is from Figure No. 10, page 45 of Entry Book E-75. UAREP data is based on Appendix VI-11 and VI-12. HLA figures for benign tumors are lower than UAREP's because they are based on animals with only a benign tumor whereas UAREP figures include mice with both a benign and malignant tumor.

APPENDIX VI-15
COMPARISON OF COMPUTATIONS BY UAREP AND HLA OF PROBABILITIES OF TUMOR
INCIDENCE IN MALE AND FEMALE MICE RECEIVING ASPARTAME
OR SERVING AS CONTROLS

Group	Males				Significance P<0.05 HLA UAREP	Females				Significance P<0.05 HLA UAREP
	HLA P	UAREP P	HLA [N]	UAREP [N]		HLA P	UAREP P	HLA [N]	UAREP [N]	
Any Tumor										
1	71.1	69.2	23.9	23.1		54.0	78.3	38.9	35.8	
2	31.0 ^S	37.4	12.9	8.0	1vs2	42.2	67.2	19.0	14.9	
3	41.2	45.6	14.6	13.2		32.8	62.5	21.3	16.0	
4	67.8	70.4	11.8	9.9		60.0	67.2	20.0	16.4	
Benign Tumors										
1	30.7	56.8	19.5	21.1		29.7	66.2	33.7	30.2	
2	10.5	37.4	9.5	8.0		21.8	61.1	18.3	14.7	
3	19.5	40.0	15.4	10.0		17.1	52.5	17.5	13.3	
4	19.1	38.5	10.5	7.8		42.5	42.8	16.5	14.0	
All Malignant Tumors										
1	46.0	37.0	23.9	16.2		29.5	38.7	37.3	31.0	
2	21.9	0	13.7	0		25.3	17.2	15.8	11.6	
3	24.8	9.3	12.1	21.5		18.9	24.1	21.2	16.6	
4	60.1	48.2	10.0	8.3		27.0	40.0	18.5	12.5	
Primary Lung Tumors										
1	53.8	52.6	18.6	16.7		21.3	29.3	32.9	23.9	
2	10.5 ^S	18.2	9.5	5.5	1vs2	20.5	25.5	19.5	15.7	
3	19.5 ^S	31.0	15.4	12.9	1vs3	12.0	21.6	16.7	13.9	
4	32.5	38.5	9.2	7.8		24.4	31.3	16.4	12.8	
Lymphoreticular Tumors										
1	11.1	16.0	18.0	12.5		17.6	21.7	39.8	32.3	
2	0	0	0	0		20.9	17.2	14.4	11.6	
3	0	0	0	0		7.4	5.6	13.5	17.8	
4	16.4	21.3	12.2	9.4		22.1	30.5	18.1	13.1	
Vascular Tumors										
1	10.1	15.4	29.7	25.9		22.3	26.4	31.4	22.7	
2	6.2	6.1	16.1	16.4		7.1	13.3	14.1	7.5	
3	10.0	18.2	10.0	5.5		12.4	24.1	24.2	16.6	
4	16.6	18.2	6.0	5.5		18.1	25.0	11.0	8.0	

^S HLA found statistically significant decrease in tumor incidence for "any tumor and "primary lung tumors"

Only group comparisons which are statistically significant are shown under the appropriate columns

P= calculated probability, X100, of developing a tumor during the total test period

[N]=estimate of "effective number" of animals on test over the entire period [number of tumor bearing mice/P]

APPENDIX VI-16A

COMPARISON OF SUMMARIES OF UAREP AND EPL HISTOPATHOLOGIC DIAGNOSES ON NON-NEOPLASTIC CHANGES FOR MALE MICE TREATED WITH ASPARTAME OR SERVING AS CONTROL FOR 104 WEEKS (E-75, FIGURE NO. 12, PAGES 47-53)

	Group 1		Group 2		Group 3		Group 4	
	UAREP	EPL	UAREP	EPL	UAREP	EPL	UAREP	EPL
<u>Adrenal</u>	16	16	9	9	9	9	5	5
Nodular hyper- plasia	1	0	0	0	1	0	0	0
Subcapsular hyper- plasia	5	14	6	4	4	4	1	2
Amyloid	10	9	5	5	3	3	5	5
<u>Thyroid</u>	15	17	1	1	3	8 ^a	2	5
Amyloid	1	7	0	0	0	0	1	3
Inflammation	0	2	0	0	0	0	0	1
Hyperplasia	0	0	0	0	0	0	0	0
<u>Pituitary</u>	15	16	8	8	4	4	5	5
<u>Heart</u>	17	17	9	9	10	10	6	6
Inflammation	3	7	1	2	8	5	0	0
Amyloid	5	5	3	3	0	0	2	4
Fibrosis	0	3	8	0	5	1	3	0
Thrombosis	2	2	0	0	0	0	0	0

^a discrepancy with Figure No. 12, page 47, E-75. Data counted from Table No. 8, page 146, E-75 shows 8 animals with thyroid diagnoses for sacrificed animals, whereas Figure No. 12 shows a total of 4.

Appendix VI-16A
(cont'd) page 2

	<u>Group 1</u>		<u>Group 2</u>		<u>Group 3</u>		<u>Group 4</u>	
	UAREP	EPL	UAREP	EPL	UAREP	EPL	UAREP	EPL
<u>Pancreas</u>	17	17	9	9	9	8	6	6
Inflammation	1	7	0	0	9	8	0	1
Atrophy/fibrosis	0	1	1	1	0	0	0	0
Amyloid	2	3	0	0	0	0	1	1
Islet cell hyperplasia	2	0	0	0	0	0	0	0
<u>Liver</u>	17	17	9	9	10	10	6	6
Vacuolation/fatty change	2	0	0	1	1	0	0	0
Inflammation	5	5	7	5	4	7	5	5
Amyloid	6	6	5	4	1	1	4	4
Necrosis	0	1	2	0	0	0	2	1
Congestion/angiectasis	1	0	1	0	1	0	1	0
Hyperplasia, nodular, diffuse/foci of change	3	1	0	0	8	1	4	0
<u>Lung</u>	16	17	9	9	10	10	6	6
Inflammation, acute abscess	2	4	0	0	0	0	0	0
Inflammation, chronic	7	12	5	6	6	6	2	2
Congestion/edema	0	0	0	0	3	0	0	0
Hemorrhage	0	0	0	0	0	0	0	0

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(cont'd) page 3

	<u>Group 1</u>		<u>Group 2</u>		<u>Group 3</u>		<u>Group 4</u>	
	UAREP	EPL	UAREP	EPL	UAREP	EPL	UAREP	EPL
<u>Kidney</u>	16	17	9	9	10	10	6	6
Inflammation	10	17	8	9	6	10	5	5
Amyloid	8	9	7	6	3	3	6	4
Tubular dilatation	0	1	6	1	0	1	2	0
<u>Spleen</u>	17	16	9	9	10	10	5	5
Extramedullary hematopoiesis	1	7	0	0	5	3	1	0
Reticuloendothelial cell hyperplasia	0	0	0	0	0	0	0	0
Lymphoid hyper- plasia	2	0	4	0	5	0	1	0
Amyloid	4	4	4	1	0	0	2	2
Congestion	0	0	0	0	1	1	0	0
<u>Lymph Node</u>	17	14	1	1	9	0	6	6
Hyperplasia/RE lymphoid	2	1	0	0	4	0	3	0
Inflammation/adenitis/ arteritis	0	0	0	0	0	0	0	1
Amyloid	3	5	1	0	0	0	1	0
<u>Salivary Gland</u>	17	17	0	0	0	0	6	6
Inflammation	0	8	0	0	0	0	3	2
Amyloid	1	7	0	0	0	0	1	1

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(cont'd) page 4

	<u>Group 1</u>		<u>Group 2</u>		<u>Group 3</u>		<u>Group 4</u>	
	UAREP	EPL	UAREP	EPL	UAREP	EPL	UAREP	EPL
<u>Small Intestine</u>	16	17	9	8	9	9	5	5
Inflammation/ ulceration	0	0	0	0	0	0	0	0
Hemorrhage	0	0	0	0	0	0	0	0
Amyloid	5	9	1	1	1	1	2	2
Parasites	2	0	0	0	0	0	0	0
<u>Large Intestine</u>	16	17	9	9	9	9	5	5
Hemorrhage	0	0	0	0	0	0	0	0
Parasites	5	6	1	1	1	1	0	0
Amyloid	0	0	0	0	0	0	0	0
<u>Stomach</u>	17	17	9	9	9	9	6	6
Inflammation	0	2	2	0	0	0	0	0
Ulceration	0	2	0	0	0	0	0	0
Amyloid	1	2	0	0	0	0	3	3
Hyperplasia	0	0	0	0	1	0	0	0
<u>Mesentery</u>	6	0	4	0	10	0	5	0
Inflammation	1	0	0	0	0	0	0	0
<u>Skin</u>	11	0	0	0	1	0	6	0
Inflammation	0	0	0	0	0	0	0	0
Parasites	1	0	0	0	0	0	0	0

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(cont'd) page 5

	<u>Group 1</u>		<u>Group 2</u>		<u>Group 3</u>		<u>Group 4</u>	
	<u>UAREP</u>	<u>EPL</u>	<u>UAREP</u>	<u>EPL</u>	<u>UAREP</u>	<u>EPL</u>	<u>UAREP</u>	<u>EPL</u>
<u>Gall Bladder</u>	14	14	7	7	9	10	4	1
Inflammation	0	0	0	0	0	0	0	0
<u>Skeletal Muscle</u>	16	17	9	0	10	0	6	6
Inflammation	1	0	0	0	0	0	0	0
<u>Bone Marrow</u>	17	17	1	0	4	0	6	6
Hematogenic activity	2	17	0	0	0	0	0	6
Inflammation	0	0	0	0	0	0	0	0
<u>Bone</u>	17	17	0	0	4	0	6	6
<u>Brain</u>	17	17	9	9	10	9	6	6
Mineral deposition	1	3	2	2	1	1	1	1
Inflammation	0	0	0	0	0	0	0	0
Hemorrhage	0	0	0	0	0	0	0	0
<u>Eye</u>	17	17	0	0	0	0	5	5
Inflammation	0	0	0	0	0	0	0	0
Retinal degeneration	0	3	0	0	0	0	0	1
<u>Urinary Bladder</u>	17	17	8	8	10	10	6	6
Inflammation	0	8	1	5	0	5	0	5
Epithelial hyperplasia	2	0	0	0	0	0	0	0
Squamous metaplasia	0	0	0	0	0	0	0	0

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(cont'd) page 6

	<u>Group 1</u>		<u>Group 2</u>		<u>Group 3</u>		<u>Group 4</u>	
	<u>UAREP</u>	<u>EPL</u>	<u>UAREP</u>	<u>EPL</u>	<u>UAREP</u>	<u>EPL</u>	<u>UAREP</u>	<u>EPL</u>
<u>Prostate</u>	17	15	8	3	10	7	5	3
Inflammation	0	1	0	0	0	0	1	1
<u>Testis</u>	17	17	7	8	10	9	6	6
Amyloid	0	6	1	1	2	2	3	3
Hypospermatogenesis/ atrophy	4	11	2	4	2	5	3	3
Inflammation	0	0	0	0	1	1	0	0
Interstitial cell hyperplasia	1	0	7	0	2	0	5	0
<u>Seminal Vesicle</u>	17	17	8	9	10	10	6	6
Inflammation	0	3	0	0	0	2	1	1

The numbers opposite each organ show total number of animals from which sections were examined.

APPENDIX VI-16B

COMPARISON OF SUMMARIES OF UAREP AND EPL HISTOPATHOLOGIC DIAGNOSES ON NON-NEOPLASTIC CHANGES FOR FEMALE MICE TREATED WITH ASPARTAME OR SERVING AS CONTROL FOR 104 WEEKS (E-75, FIGURE NO. 12, PAGES 47-53)

	<u>Group 1</u>		<u>Group 2</u>		<u>Group 3</u>		<u>Group 4</u>	
	UAREP	EPL	UAREP	EPL	UAREP	EPL	UAREP	EPL
<u>Adrenal</u>	24	25	10	10	10	10	9	9
Nodular hyperplasia	2	0	0	0	0	0	0	0
Subcapsular hyper- plasia	23	25	9	10	9	10	8	9
Amyloid	8	8	4	1	4	4	1	2
<u>Thyroid</u>	15	21	7	8	10	10	8	9
Amyloid	4	4	1	2	2	2	0	0
Inflammation	4	4	0	0	0	2	0	0
Hyperplasia	0	0	0	0	0	0	0	0
<u>Pituitary</u>	18	18	3	3	5	4	1	2
<u>Heart</u>	26	24	11	11	11	11	10	10
Inflammation	3	3	2	3	1	1	1	2
Amyloid	8	6	1	3	1	2	1	1
Fibrosis	7	0	2	0	7	0	8	0
Thrombosis	0	0	1	1	0	0	0	0

Appendix VI- 16B
(cont'd) page 2

	<u>Group 1</u>		<u>Group 2</u>		<u>Group 3</u>		<u>Group 4</u>	
	UAREP	EPL	UAREP	EPL	UAREP	EPL	UAREP	EPL
<u>Pancreas</u>	26	25	11	11	11	10	7	10
Inflammation	1	3	0	3	1	2	1	4
Atrophy/fibrosis	2	2	0	0	0	0	0	0
Amyloid	1	1	0	0	0	0	0	0
Islet cell hyperplasia	0	0	0	0	0	0	0	0
<u>Liver</u>	26	26	11	11	11	11	10	10
Vacuolation/fatty change	1	0	0	0	2	0	0	0
Inflammation	21	19	4	9	7	8	6	8
Amyloid	5	6	2	2	4	2	1	1
Necrosis	3	2	0	0	0	0	1	1
Congestion/angiectasis	2	0	0	0	0	0	1	1
Hyperplasia, nodular, diffuse/Foci of change	23	1	2	0	1	1	2	0
<u>Lung</u>	26	26	11	11	10	10	9	10
Inflammation, acute abscess	1	3	0	1	0	4	0	1
Inflammation, chronic	8	19	3	9	4	2	6	7
Congestion/edema	6	0	0	0	0	0	0	0
Hemorrhage	0	0	0	0	0	0	0	0

Appendix VI-16B
(cont'd) page 3

	<u>Group 1</u>		<u>Group 2</u>		<u>Group 3</u>		<u>Group 4</u>	
	UAREP	EPL	UAREP	EPL	UAREP	EPL	UAREP	EPL
<u>Kidney</u>	25	26	11	11	11	11	10	10
Inflammation	20	26	10	11	11	11	9	10
Amyloid	21	10	4	5	6	6	7	6
Tubular dilatation	4	0	0	0	0	0	2	0
<u>Spleen</u>	26	26	11	11	11	11	10	10
Extramedullary hematopoiesis	9	9	7	4	2	7	7	0
Reticuloendothelial cell hyperplasia	3	2	1	0	1	1	0	0
Lymphoid hyper- plasia	7	4	4	0	4	0	3	3
Amyloid	3	1	2	2	0	0	1	1
Congestion	1	0	0	0	0	0	0	0
<u>Lymph Node</u>	24	22	11	6	7	2	10	8
Hyperplasia/RE lymphoid	14	0	6	2	2	1	7	0
Inflammation/adenitis/ arteritis	0	0	0	0	0	0	0	0
Amyloid	2	0	0	1	0	0	0	0
<u>Salivary Gland</u>	24	23	0	0	5	1	10	10
Inflammation	3	7	0	0	0	0	5	5
Amyloid	9	9	0	0	0	0	1	1

Appendix VI-16B
(cont'd) page 4

	<u>Group 1</u>		<u>Group 2</u>		<u>Group 3</u>		<u>Group 4</u>	
	UAREP	EPL	UAREP	EPL	UAREP	EPL	UAREP	EPL
<u>Small Intestine</u>	26	25	11	11	10	10	9	9
Inflammation/ulcer- ation	2	0	0	0	0	0	0	0
Hemorrhage	0	0	0	0	0	0	0	0
Amyloid	5	6	3	4	4	3	2	2
Parasite	0	0	0	0	0	0	0	0
<u>Large Intestine</u>	21	23	11	10	10	11	10	10
Hemorrhage	0	0	0	0	0	0	0	0
Parasites	3	2	2	3	0	1	2	2
Amyloid	0	0	0	0	0	0	0	0
<u>Stomach</u>	25	26	11	10	10	11	8	10
Inflammation	0	2	0	1	0	1	2	0
Ulceration	0	0	0	1	0	0	0	0
Amyloid	1	2	0	0	1	0	0	0
Hyperplasia	0	0	0	0	0	0	0	0
<u>Mesentery</u>	26	0	11	0	11	0	7	0
Inflammation	1	0	0	0	0	0	0	0
<u>Skin</u>	26	0	3	0	7	0	10	0
Inflammation	0	0	0	0	0	0	0	0
Parasite	2	0	0	0	0	0	0	0

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(cont'd) page 5

	<u>Group 1</u>		<u>Group 2</u>		<u>Group 3</u>		<u>Group 4</u>	
	UAREP	EPL	UAREP	EPL	UAREP	EPL	UAREP	EPL
<u>Gall Bladder</u>	19	25	7	8	10	10	8	7
Inflammation	0	0	1	1	0	0	0	0
<u>Skeletal Muscle</u>	26	26	11	0	10	0	10	10
Inflammation	1	2	0	0	0	0	0	0
<u>Bone Marrow</u>	25	26	9	0	2	0	10	9
Hematogenic activity	20	26	0	0	2	0	0	9
Inflammation	0	0	0	0	0	0	0	0
<u>Bone</u>	24	26	9	0	2	0	10	10
<u>Brain</u>	26	26	9	9	11	11	10	10
Mineral deposition	3	7	1	4	2	5	1	1
Inflammation	0	0	0	0	0	0	0	0
Hemorrhage	0	0	0	0	0	0	0	0
<u>Eye</u>	21	22	0	0	0	0	8	10
Inflammation	0	3	0	0	0	0	0	0
Retinal degeneration	0	7	0	0	0	0	0	1
<u>Urinary Bladder</u>	26	26	10	10	10	10	9	9
Inflammation	2	17	1	7	3	5	1	4
Epithelial hyper- plasia	2	0	0	0	0	0	0	0
Squamous metaplasia	2	0	0	0	0	0	0	0

Appendix VI-16B
(cont'd) page 6

	<u>Group 1</u>		<u>Group 2</u>		<u>Group 3</u>		<u>Group 4</u>	
	<u>UAREP</u>	<u>EPL</u>	<u>UAREP</u>	<u>EPL</u>	<u>UAREP</u>	<u>EPL</u>	<u>UAREP</u>	<u>EPL</u>
<u>Vagina</u>	24	24	9	7	8	8	8	9
Inflammation	1	1	1	0	4	1	0	1
<u>Ovary</u>	24	26	10	8	10	11	9	10
Inflammation	8	1	1	1	1	1	0	0
Amyloid	15	10	3	4	4	3	3	4
Cyst	15	15	5	7	6	10	6	8
<u>Uterus</u>	26	26	11	10	10	11	10	10
Endometritis	2	1	0	0	1	1	0	0
Hyperplasia	4	4	7	4	5	9	10	0
Hemorrhage/thrombosis	6	4	2	3	5	8	2	1
Angiectasis	0	0	0	0	1	0	0	0

The numbers opposite each organ show total number of animals from which sections were examined.

APPENDIX VI-16C

COMPARISON OF SUMMARIES OF UAREP AND EPL HISTOPATHOLOGIC DIAGNOSES ON
NON-NEOPLASTIC CHANGES FOR MALE MICE TREATED WITH ASPARTAME OR SERVING
AS CONTROLS WHICH WERE FOUND DEAD OR SACRIFICED IN A MORIBUND CONDITION
(E-75, FIGURE NO. 12A, PAGES 54-61)

	<u>Group 1</u>		<u>Group 2</u>		<u>Group 3</u>		<u>Group 4</u>	
	UAREP	EPL	UAREP	EPL	UAREP	EPL	UAREP	EPL
<u>Adrenal</u>	39	41	4	4	14	14	21	21
Nodular hyper- plasia	0	6	1	0	3	0	0	0
Subcapsular hyper- plasia	14	7	2	2	6	6	11	6
Amyloid	22	21	2	2	10	10	14	14
<u>Thyroid</u>	22	28	0	0	2	2	5	9
Amyloid	5	7	0	0	0	0	1	1
Inflammation	0	0	0	0	0	0	0	0
Hyperplasia	0	2	0	0	0	0	0	0
<u>Pituitary</u>	28	30	3	3	9	10	17	17
<u>Heart</u>	48	48	5	5	15	15	21	21
Inflammation	5	6	2	2	1	1	1	3
Amyloid	16	19	0	1	4	8	3	5
Fibrosis	21	1	2	0	12	0	16	0
Thrombosis	2	3	1	1	3	2	3	3

Appendix VI-16C
(cont'd) page 2

	<u>Group 1</u>		<u>Group 2</u>		<u>Group 3</u>		<u>Group 4</u>	
	UAREP	EPL	UAREP	EPL	UAREP	EPL	UAREP	EPL
<u>Pancreas</u>	42	45	7	6	14	12	21	21
Inflammation	3	2	0	1	1	1	2	1
Atrophy/fibrosis	0	1	0	0	0	0	0	0
Amyloid	0	1	1	0	0	2	0	0
Islet cell hyper- plasia	0	0	0	0	0	0	0	1
<u>Liver</u>	46	48	8	4	19	19	21	21
Vacuolation/fatty change	0	0	1	0	2	0	0	0
Inflammation	9	5	8	0	8	4	7	3
Amyloid	19	19	5	3	13	12	9	9
Necrosis	1	1	1	0	1	0	0	0
Congestion/angiectasis	20	0	2	0	13	0	11	0
Hyperplasia, nodular diffuse/Foci of change	7	0	4	0	17	1	0	0
<u>Lung</u>	46	46	14	14	16	16	20	20
Inflammation, acute abscess	1	1	0	2	0	1	1	3
Inflammation, chronic	13	14	2	5	1	1	6	3
Congestion/edema	33	0	12	0	10	0	11	0
Hemorrhage	4	0	1	0	0	0	0	0

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(cont'd) page 3

	<u>Group 1</u>		<u>Group 2</u>		<u>Group 3</u>		<u>Group 4</u>	
	UAREP	EPL	UAREP	EPL	UAREP	EPL	UAREP	EPL
<u>Kidney</u>	48	48	12	12	19	19	21	21
Inflammation	34	32	9	11	13	17	12	15
Amyloid	32	25	10	11	17	17	14	11
Tubular dilatation	9	2	3	2	2	1	7	1
<u>Spleen</u>	39	41	6	5	14	14	20	20
Extramedullary hematopoiesis	4	1	1	0	4	1	1	0
Reticuloendothelial cell hyperplasia	0	0	1	1	0	0	0	0
Lymphoid hyper- plasia	3	0	0	0	0	0	2	0
Amyloid	14	9	2	1	7	6	8	3
Congestion	2	0	1	0	0	0	0	0
<u>Lymph Node</u>	29	31	4	1	13	1	17	16
Hyperplasia/RE lymphoid	2	0	0	0	0	0	2	0
Inflammation/adenitis/ arteritis	0	0	0	0	0	0	0	0
Amyloid	7	4	0	0	1	1	1	2
<u>Salivary Gland</u>	44	43	2	0	1	0	20	20
Inflammation	7	8	0	0	0	0	2	3
Amyloid	9	15	0	0	0	0	0	3

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(cont'd) page 4

	<u>Group 1</u>		<u>Group 2</u>		<u>Group 3</u>		<u>Group 4</u>	
	UAREP	EPL	UAREP	EPL	UAREP	EPL	UAREP	EPL
<u>Small Intestine</u>	45	44	6	5	19	12	16	19
Inflammation/ ulceration	0	0	0	0	0	0	0	0
Hemorrhage	0	0	0	0	0	0	1	2
Amyloid	12	13	2	2	5	2	4	6
Parasite	0	0	0	0	0	0	0	0
<u>Large Intestine</u>	44	44	4	4	13	13	20	20
Hemorrhage	0	0	0	0	0	0	0	0
Parasites	14	16	1	1	2	2	3	3
Amyloid	1	0	0	0	1	0	0	0
<u>Stomach</u>	47	47	10	10	16	16	21	21
Inflammation	1	0	0	0	0	0	0	0
Ulceration	0	6	0	2	0	0	0	2
Amyloid	8	7	0	0	0	4	1	3
Hyperplasia	0	0	0	0	0	0	0	0
<u>Mesentery</u>	27	0	9	0	16	0	19	0
Inflammation	0	0	0	0	0	0	0	0

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(cont'd) page 5

	Group 1		Group 2		Group 3		Group 4	
	UAREP	EPL	UAREP	EPL	UAREP	EPL	UAREP	EPL
<u>Skin</u>	12	0	2	0	1	0	19	1 ^a
Inflammation	2	0	0	0	0	0	0	0
Parasite	2	0	0	0	0	0	0	0
<u>Gall Bladder</u>	8	8	2	3	12	11	14	13
Inflammation	0	0	0	0	0	0	0	0
<u>Skeletal Muscle</u>	47	44	6	1	14	0	21	20
Inflammation	2	1	0	0	0	0	0	0
<u>Bone</u>	45	46	1	0	9	0	21	21
<u>Bone Marrow</u>	46	46	1	0	7	0	21	21
Hematogenic activity	10	43	0	0	0	0	0	20
Inflammation	0	0	0	0	0	0	0	0
<u>Brain</u>	47	46	32	22	24	25	21	21
Mineral deposition	4	4	1	2	3	4	3	5
Inflammation	1	1	0	0	0	0	0	0
Hemorrhage	0	0	0	0	0	0	0	0

^a discrepancy with Figure No. 12A, page 54, E-75. Skin is not listed in this table, whereas Table No. 8, page 184 E-75 shows a diagnosis for skin on Animal Number 99-826, Group 4 Male deaths.

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(cont'd) page 6

	<u>Group 1</u>		<u>Group 2</u>		<u>Group 3</u>		<u>Group 4</u>	
	UAREP	EPL	UAREP	EPL	UAREP	EPL	UAREP	EPL
<u>Eye</u>	42	43	0	0	1	1	21	21
Inflammation	1	2	0	0	0	0	1	3
Retinal degeneration	0	2	0	0	0	0	0	1
<u>Urinary Bladder</u>	44	46	21	22	23	23	21	19
Inflammation	4	11	1	6	1	8	6	4
Epithelial hyperplasia	4	0	2	1	0	0	1	1
Squamous metaplasia	1	0	0	0	0	0	1	0
<u>Prostate</u>	41	29	13	3	20	9	19	16
Inflammation	5	2	1	0	3	1	1	0
<u>Testis</u>	46	46	5	5	14	14	19	20
Amyloid	15	19	3	3	5	10	8	7
Hypospermatogenesis/ atrophy	15	22	2	3	5	9	0	10
Inflammation	1	0	2	1	3	1	0	2
Interstitial cell hyperplasia	24	1	0	0	4	0	11	0
<u>Seminal Vesicle</u>	45	43	7	7	19	15	20	20
Inflammation	1	4	0	0	1	3	1	1

The numbers opposite each organ show total number of animals from which sections were examined.

APPENDIX VI-16D

COMPARISON OF SUMMARIES OF UAREP AND EPL HISTOPATHOLOGIC DIAGNOSES ON
NON-NEOPLASTIC CHANGES FOR FEMALE MICE TREATED WITH ASPARTAME OR SERVING
AS CONTROLS WHICH WERE FOUND DEAD OR SACRIFICED IN A MORIBUND CONDITION

(E-75, FIGURE NO. 12A, PAGES 54-61)

	<u>Group 1</u>		<u>Group 2</u>		<u>Group 3</u>		<u>Group 4</u>	
	<u>UAREP</u>	<u>EPL</u>	<u>UAREP</u>	<u>EPL</u>	<u>UAREP</u>	<u>EPL</u>	<u>UAREP</u>	<u>EPL</u>
<u>Adrenal</u>	37	38	2	1	11	12	20	20
Nodular hyperplasia	0	0	0	0	1	0	0	0
Subcapsular hyper- plasia	33	28	1	1	7	7	13	11
Amyloid	24	20	0	0	9	9	11	11
<u>Thyroid</u>	7	8	0	0	7	8	10	13
Amyloid	4	3	0	0	1	5	5	4
Inflammation	1	1	0	0	0	1	1	1
Hyperplasia	0	0	0	0	1	0	0	0
<u>Pituitary</u>	27	34	0	0	6	7	7	9
<u>Heart</u>	40	40	2	2	14	13	20	20
Inflammation	5	5	0	0	6	3	1	1
Amyloid	8	11	2	2	5	4	4	6
Fibrosis	24	0	1	0	10	0	12	1
Thrombosis	2	2	0	0	0	1	1	1

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(cont'd) page 2

	<u>Group 1</u>		<u>Group 2</u>		<u>Group 3</u>		<u>Group 4</u>	
	<u>UAREP</u>	<u>EPL</u>	<u>UAREP</u>	<u>EPL</u>	<u>UAREP</u>	<u>EPL</u>	<u>UAREP</u>	<u>EPL</u>
<u>Pancreas</u>	36	35	3	1	16	15	20	20
Inflammation	2	2	0	0	2	2	0	2
Atrophy/fibrosis	1	1	0	0	0	0	0	0
Amyloid	1	1	0	0	1	1	1	2
Islet cell hyper- plasia	0	0	0	0	0	0	0	0
<u>Liver</u>	39	39	3	3	16	16	20	20
Vacuolation/fatty change	1	0	0	0	3	0	0	0
Inflammation	21	8	1	0	10	8	2	4
Amyloid	22	16	2	2	13	12	9	9
Necrosis	6	0	0	0	2	1	1	0
Congestion/ angiectasis	14	1	0	0	3	0	10	0
Hyperplasia, nodular diffuse/Foci of change	26	0	0	0	12	1	0	0
<u>Lung</u>	39	39	12	12	19	19	20	20
Inflammation, acute abscess	1	5	1	3	0	0	1	3
Inflammation, chronic	11	5	5	3	3	6	6	3
Congestion/edema	23	0	3	0	14	0	12	0
Hemorrhage	0	0	0	0	0	0	0	0

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(cont'd) page 3

	<u>Group 1</u>		<u>Group 2</u>		<u>Group 3</u>		<u>Group 4</u>	
	UAREP	EPL	UAREP	EPL	UAREP	EPL	UAREP	EPL
<u>Kidney</u>	40	40	8	8	16	16	20	20
Inflammation	27	21	6	3	15	13	12	10
Amyloid	28	20	6	4	14	13	12	11
Tubular dilatation	7	1	0	0	0	1	6	0
<u>Spleen</u>	39	37	4	4	16	16	20	19
Extramedullary hematopoiesis	5	1	0	0	4	3	0	0
Reticuloendothelial cell hyperplasia	3	2	0	0	5	2	2	1
Lymphoid hyperplasia	6	6	1	2	3	1	2	4
Amyloid	20	6	2	1	7	6	5	2
Congestion	0	0	0	0	2	0	0	0
<u>Lymph Node</u>	34	30	4	3	11	2	17	15
Hyperplasia/RE lymphoid	10	0	0	0	3	0	4	2
Inflammation/adenitis/ arteritis	1	1	0	0	1	1	0	0
Amyloid	7	2	0	0	1	1	2	0
<u>Salivary Gland</u>	28	25	1	1	2	2	19	19
Inflammation	4	7	0	0	1	0	7	5
Amyloid	12	13	0	1	0	1	6	7

Appendix VI-16D
(cont'd) page 4

	<u>Group 1</u>		<u>Group 2</u>		<u>Group 3</u>		<u>Group 4</u>	
	UAREP	EPL	UAREP	EPL	UAREP	EPL	UAREP	EPL
<u>Small Intestine</u>	36	37	4	3	15	11	19	17
Inflammation/ ulceration	1	0	0	0	1	0	0	0
Hemorrhage	0	0	0	0	1	1	0	1
Amyloid	18	12	1	2	9	7	6	7
Parasite	1	0	0	0	0	0	0	0
<u>Large Intestine</u>	38	39	3	1	14	15	20	19
Hemorrhage	0	0	0	0	0	0	0	0
Parasites	4	6	1	1	1	1	3	5
Amyloid	2	0	0	0	1	0	0	0
<u>Stomach</u>	39	40	6	5	16	16	20	19
Inflammation	3	0	0	0	0	0	3	0
Ulceration	0	2	0	0	0	1	0	0
Amyloid	3	4	0	0	2	2	3	0
Hyperplasia	0	0	0	0	0	0	0	0
<u>Mesentery</u>	36	0	1	0	16	2	16	0
Inflammation	0	0	0	0	2	2	0	0

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(cont'd) page 5

	<u>Group 1</u>		<u>Group 2</u>		<u>Group 3</u>		<u>Group 4</u>	
	UAREP	EPL	UAREP	EPL	UAREP	EPL	UAREP	EPL
<u>Skin</u>	38	0	1	0	5	0	21	1 ^a
Inflammation	1	0	0	0	0	0	0	0
Parasite	0	0	0	0	0	0	0	0
<u>Gall Bladder</u>	24	30	2	2	10	10	14	14
Inflammation	1	0	0	0	0	0	0	0
<u>Skeletal Muscle</u>	40	38	2	0	12	0	21	20
Inflammation	5	2	0	0	0	0	0	0
<u>Bone Marrow</u>	38	39	1	0	3	0	21	20
Hematogenic activity	9	35	1	0	0	0	6	18
Inflammation	0	0	0	0	0	0	0	0
<u>Bone</u>	38	39	1	0	3	0	21	21
<u>Brain</u>	40	40	19	18	20	20	21	21
Mineral deposition	4	2	3	5	0	3	2	2
Inflammation	1	1	0	0	0	1	0	0
Hemorrhage	0	0	0	0	0	0	0	0

^a discrepancy with Figure No. 12A, page 54, E-75. Skin is not listed in this table, whereas Table No. 8 page 295, E-75 shows a diagnosis for skin on Animal Number 99-889, Group 4 Female deaths

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(cont'd) page 6

	Group 1		Group 2		Group 3		Group 4	
	UAREP	EPL	UAREP	EPL	UAREP	EPL	UAREP	EPL
<u>Eye</u>	35	34	0	0	1	1	19	20
Inflammation	0	1	0	0	0	0	0	0
Retinal degeneration	0	0	0	0	0	0	0	1
<u>Urinary Bladder</u>	33	35	17	18	16	16	13	15
Inflammation	4	14	1	3	4	8	4	7
Epithelial hyper- plasia	1	0	1	0	3	0	0	0
Squamous metaplasia	0	0	0	0	0	0	0	0
<u>Vagina</u>	19	20	1	1	13	8	12	14
Inflammation	1	1	1	0	4	2	0	1
<u>Ovary</u>	33	31	1	3	9	10	16	16
Inflammation	3	2	0	0	3	0	0	0
Amyloid	13	12	1	1	6	8	5	7
Cyst	15	16	0	2	3	4	6	9
<u>Uterus</u>	38	36 ^b	12	12	18	18	21	21
Endometritis	5	4	0	0	2	2	2	0
Hyperplasia	16	3	2	1	5	4	8	0
Hemorrhage/thrombosis	7	8	2	3	2	1	2	4
Angiectasis	4	0	0	0	0	0	0	0

^b discrepancy with Figure No. 12A, page 59, E-75. Data counted from Table No. 8, pages 215, 216, and 217, E-17 show 36 animals with uterine diagnoses from Group 1 Female deaths, whereas Figure No. 12A shows a total of 37.

The numbers opposite each organ show total number of animals from which sections were examined.

APPENDIX VI-17
SIGNIFICANT DISCREPANCIES BETWEEN HISTOPATHOLOGIC DIAGNOSES
BY UAREP AND EPL ON E-75

Group Set	Animal No.	Path No.	Organ	EPL Diagnosis	UAREP Diagnosis
1M	99-538	100-019	Testis	0	Interstitial cell hyperplasia 3
1M	99-540	100-021	Liver	X	Congestion/angiectasis 4
1M	99-541	100-022	Testis	X	Interstitial cell hyperplasia 3
1M	99-544	100-025	Lung	X	Congestion/edema/hemorrhage 4
1M	99-548	100-028	Lung	X	Chronic inflammation 3
1M	99-559	100-006	Lung	Adenoma	0
1M	99-563	100-007	Testis	Hypospermatogenesis 3	X
1M	99-562	100-037	Lung	X	Chronic inflammation 3
1M	99-569	100-040	Skin	0	Dermatitis 4
1M	99-572	100-043	Liver	Hepatoma, malignant	Hepatoma, benign
1M	99-573	100-010	Lung	0	Chronic inflammation 3
1M	99-574	100-044	Adrenal	Cortical carcinoma	0
1M	99-576	100-012	Liver	Hepatoma, malignant	Hyperplasia
1M	99-577	100-045	Heart	Myocarditis 3	0
1M	99-579	100-046	Testis	Hypospermatogenesis 4	Interstitial cell hyperplasia 3
1M	99-580	100-047	Testis	X	Interstitial cell hyperplasia 3
1M	99-583	100-014	Testis	Hypospermatogenesis 3	Interstitial cell hyperplasia 4
1M	99-595	100-016	Liver	Hepatoma, malignant	Hepatoma, benign
1M	99-597	100-058	Liver Lung	Angiosarcoma X	Angioma Congestion/edema 4
1M	99-598	100-059	Skin	0	Angioma
1M	99-599	100-061	Testis	Hypospermatogenesis 4	Interstitial cell hyperplasia 2
1M	99-602	100-063	Urinary bladder	Acute cystitis 3	X
1M	99-605	100-066	Lung	X	Congestion/edema 4
1F	99-609	100-189	Liver	X	Nodular hyperplasia 3
1F	99-611	100-191	Spleen	Extramedullary hemato- poiesis 3	Lymphoid depletion 4
1F	99-613	100-193	Liver	X	Nodular hyperplasia 3
1F	99-615	100-162	Uterus	Glandular dilatation 4	Polyp
1F	99-616	100-195	Pancreas	X	Focal adenoma
1F	99-617	100-163	Uterus	Glandular dilatation 4	Polyp
1F	99-618	100-196	Uterus	X	Endometritis 3
1F	99-621	100-165	Liver	0	Nodular hyperplasia 3

X - indicates section was unremarkable
0 - indicates that no comparable diagnosis was recorded
1-5 degrees of severity of diagnosis as follows:
1-minimal
2-slight
3-moderate
4-moderately severe/high
5-severe/high

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continued, page 2

Group Set	Animal No.	Path No.	Organ	EPL Diagnosis	UAREP Diagnosis
1F	99-622	100-197	Liver	0	Nodular hyperplasia 3
1F	99-625	100-199	Liver	0	Inflammation 3
1F	99-626	100-200	Adrenal Lung	0 Adenoma	Cortical hyperplasia 3 Adenoma and Adenocarcinoma
1F	99-633	100-171	Ovary	Atrophy 3	Luteoma
1F	99-636	100-204	Liver	0	Nodular hyperplasia 3
1F	99-643	100-209	Skeletal muscle	X	Inflammation 3
1F	99-648	100-175	Liver Lung	Nodular hyperplasia 2 Adenoma	Hepatoma, benign Adenocarcinoma
1F	99-655	100-180	Uterus Lymph node	Sarcoma X	0 Hyperplasia 4
1F	99-658	100-214	Ovary	Acute oophoritis 3	0
1F	99-669	100-222	Eye	Dacryoadenitis 3	Lacrimal gland papillary adenoma
1F	99-670	100-184	Liver Ovary	0 0	Hepatoma, benign Inflammation 3
1F	99-677	100-187	Heart Ovary	0 0	Inflammation 3 Luteoma
2M	99-685	100-104	Heart	Inflammation 1	Inflammation 4
2M	99-693	100-111	Kidney	Amyloid	0
2M	99-700	100-105	Testis	0	Inflammation 3
2M	99-701	100-101	Testis	X	Interstitial cell hyperplasia 3
2F	99-724	100-274	Uterus	Glandular dilatation 4	Polyp
2F	99-726	100-262	Gallbladder Vagina Uterus	Lymphosarcoma Carcinoma 0	Lymphoid infiltrate 5 Hyperplasia 5 Inflammation 3
2F	99-731	100-279	Lung	Adenocarcinoma	Alveolar adenoma
2F	99-748	100-268	Spleen	X	Extramedullary hematopoiesis 4
2F	99-751	100-269	Heart	X	Fibrosis 3
3M	99-752	100-151	Liver	Nodular hyperplasia 3	Hepatoma, malignant
3M	99-754	100-137	Liver Prostate	0 0	Nodular hyperplasia 3 Prostatitis 4
3M	99-755	100-138	Liver	0	Nodular hyperplasia 3
3M	99-756	100-139	Spleen	X	Extramedullary hematopoiesis 4
3M	99-757	100-128	Heart	0	Fibrosis 3
3M	99-760	100-141	Liver Lung	Bile duct carcinoma Metastatic Tumor	Metastatic carcinoma Adenocarcinoma
3M	99-763	100-154	Lung	X	Congestion/edema 4
3M	99-781	100-133	Spleen	Angiosarcoma	Angioma
3M	99-784	100-135	Stomach Testis	Carcinoma Arteritis 4	Hyperplasia 3 Inflammation 1
3F	99-800	100-306	Ovary Liver	0 0	Luteoma Nodular hyperplasia 3
3F	99-803	100-307	Lung	X	Alveolar adenoma

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continued, page 3

Group Set	Animal No.	Path No.	Organ	EPL Diagnosis	UAREP Diagnosis
3F	99-804	100-295	Kidney Uterus	Interstitial nephritis 4 Glandular hyperplasia 3	Inflammation 1 0
3F	99-805	100-296	Uterus	Glandular hyperplasia 3	0
3F	99-806	100-303	Kidney	Interstitial nephritis 4	Inflammation 1
3F	99-821	100-312	Small intestine	Hemorrhage 4	Ulceration/inflammation 5
3F	99-823	100-300	Uterus	0	Polyp
4H	99-825	100-075	Heart	Myocarditis 3	0
4H	99-826	100-076	Eye	Dacryoadenitis 3	X
4H	99-838	100-068	Pancreas Liver	Arteritis 3 Hepatoma, malignant	X Hepatoma, benign
4H	99-843	100-034	Testis	X	Interstitial cell hyperplasia 3
4H	99-849	100-033	Bladder	Carcinoma	0
4H	99-850	100-089	Heart	X	Fibrosis 3
4F	99-871	100-230	Spleen Lymph node	X Angioma	Extramedullary hematopoiesis 4 0
4F	99-877	100-248	Skeletal muscle	Sarcoma	X
4F	99-879	100-249	Lung	X	Congestion/edema 4
4F	99-888	100-253	Lung	X	Congestion/edema 4
4F	99-894	100-257	Uterus	0	Endometritis 3

APPENDIX VI-18

ANIMALS ON E-75, WHICH WERE NOT EXAMINED MICROSCOPICALLY

<u>Group</u>	<u>Mouse No.</u>	<u>Necropsy Comments</u>	<u>Tissue Taken</u>
1M	99545	Moderate autolysis	Yes ¹
	99550	Due to very advanced autolysis, no observations made. Entire animal preserved.	No
	99565	Due to very advanced autolysis, no gross observations can be made.	No
	99582	No necropsy performed due to very advanced autolysis of animal. Preserved in entirety.	No**
	99596	Animal Missing	---
	99606	Due to very advanced autolysis, no observations made. Entire animal saved.	No
	99607	Due to very advanced autolysis, no observations can be made. All organs and entire carcass saved.	Yes
2M	99681	Advanced autolysis	Yes
	99686	Body put in whole due to advanced autolysis.	No
	99696	Due to advanced autolysis, no observation made. Entire animal saved.	Yes
	99703	No accurate observations could be made due to advanced autolysis.	Yes

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(cont'd) page 2

<u>Group</u>	<u>Mouse No.</u>	<u>Necropsy Comments</u>	<u>Tissue Taken</u>
3M	99762	Moderate autolysis	Yes
	99771	No necropsy performed due to advanced autolysis. Animal preserved in entirety.	No**
4M	99824	No observations made due to advanced autolysis.	Yes
	99827	No necropsy performed due to very advanced autolysis. Animal preserved in its entirety.	No
	99828	Animal missing	---
	99832	Due to very advanced autolysis, animal preserved whole. All tissues with carcass in cup. No gross observations can be made.	No
	99833	Animal missing	---
	99834	No necropsy performed due to advanced autolysis. Animal preserved in entirety.	No**
	99836	Due to very advanced autolysis, no observations made. Entire carcass saved.	No
	99846	Animal missing	---
	99854	Due to very advanced autolysis, no observations made. Entire carcass saved.	No
	99858	No necropsy performed due to very advanced autolysis. Animal preserved in its entirety.	No

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(cont'd) page 3

<u>Group</u>	<u>Mouse No.</u>	<u>Necropsy Comments</u>	<u>Tissue Taken</u>
1F	99620	Animal missing	No
	99638	No necropsy due to very advanced autolysis. Animal preserved.	No
	99646	No necropsy performed due to very advanced autolysis of animal. Preserved in entirety.	No**
	99663	Due to advanced autolysis, no observations made but animal was preserved whole.	No**
	99664	Due to very advanced autolysis, no observations made. Entire animal preserved.	No
	99676	Due to very advanced autolysis, entire animal preserved intact with no gross observations made.	No
2F	99716	Animal missing	---
	99723	Animal missing	---
	99736	No necropsy performed due to very advanced autolysis of animal. Preserved in entirety.	No**
	99745	No necropsy performed due to very advanced autolysis. Animal preserved in its entirety.	No
3F	99794	Due to very advanced autolysis, no observations made and entire animal saved.	Yes

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(cont'd) page 4

<u>Group</u>	<u>Mouse No.</u>	<u>Necropsy Comments</u>	<u>Tissue Taken</u>
3F	99797	Necropsy not performed due to advanced autolysis.	No
	99816	No necropsy performed due to advanced autolysis.	No
	99820	Advanced autolysis.	Yes
4F	99865	No necropsy performed due to advanced autolysis. Animal preserved in entirety.	No**
	99866	No observations made due to very advanced autolysis. Mouse taken whole.	Yes
	99878	Due to advanced autolysis, no gross observations can be made.	Yes
	99880	No necropsy performed due to very advanced autolysis. Animal preserved in its entirety.	No

¹ Tissue fixed but lost. No further explanation provided.

The individual animal necropsy sheets (see Appendix VI-30 for examples) contain a column entitled "Tissues taken". The organs intended to have tissues taken at necropsy are precoded. The prosector is to check tissue removed for fixation. Whether no organs were checked on the sheet is shown by Yes or No.

**Indicates that "Carcass" was added and checked under "Tissues taken" column on the necropsy sheet.

APPENDIX VI-19

DEVIATIONS FROM PROTOCOL SPECIFICATIONS FOR ORGANS EXAMINED
MICROSCOPICALLY BY GROUP, E-75

	<u>Group 1</u>		<u>Group 2</u>		<u>Group 3</u>		<u>Group 4</u>	
	M	F	M	F	M	F	M	F
Brain	-2	0	-1	-4(all)	-1	0(all)	0	0
Pituitary	-19	-13	-1	-9(12)	-10	-13(24)	-5	-20
Spinal cord	-3	-5	0	0(0)	0	0(0)	0	-1
Eye	-5	-10	0	0(0)	+1	+1(0)	-1	-1
Salivary gland	-5	-18	0	+1(0)	0	+3(0)	-1	-2
Thyroid	-20	-37	-11	-4(12)	-18	-6(24)	-13	-9
Lung	-2	-1	+11	+11(12)	+2	+5(24)	-1	-1
Heart	0	-2	+2	+1(12)	+1	0(24)	0	-1
Liver	0	-1	+1	+2(12)	+5	+3(24)	0	-1
Gall bladder	-43	-11	-2	-2(12)	-3	-4(24)	-13	-10
Spleen	-8	-3	+2	+3(12)	0	+3(24)	-2	-2
Kidney	0	0	+9	+7(12)	+5	+3(24)	0	-1
Adrenal	-8	-3	+1	-1(12)	-1	-2(24)	-1	-2
Stomach	-1	0	+7	+3(12)	+1	+3(24)	0	-2
Pancreas	-3	-6	+3	0(12)	-4	+1(24)	0	-1
Small intestine	-4	-4	+1	+2(12)	-3	-3(24)	-3	-5
Large intestine	-4	-4	+1	-1(12)	-2	+2(24)	-2	-2
Lymph node	-20	-14	+2	+9(0)	+1	+4(0)	-5	-8
Bladder	-2	-5	-2	-3(all)	-2	-5(all)	-2	-7
Testis/Ovary	-2	-9	+1	-1(12)	-1	-3(24)	-1	-1

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(cont'd) page 2

	Group 1		Group 2		Group 3		Group 4	
	M	F	M	F	M	F	M	F
Seminal vesicle/ Uterus	-5	-3	+4	+10(12)	+1	+5(24)	-1	0
Prostate/Vagina	-21	-22	-6	-4(12)	-8	-8(24)	-8	-8
Mammary gland	-2	-4	0	0(0)	0	+1(0)	-2	-5
Bone	-2	-1	0	0(0)	0	0(0)	0	0
Bone marrow	-2	-1	0	0(0)	0	0(0)	0	-2
Skeletal muscle	-4	-2	+1	0(0)	0	0(0)	-1	-1
Nerve	-4	-5	+1	0(0)	0	0(0)	-2	-1
Thymus	-65	-56	-	- (0)	-	- (0)	-27	-31
Total								
Protocol plan	72/72	72/72	12/36	12/36	24/36	24/36	36/36	36/36
Initial animals available	72	72	36	36	37	35	37	35
Animals available at termination of study	65	66	32	31	35	31	27	31

Protocol specifications were adjusted to total number of animals available when applicable.

Numbers in parentheses indicate total sections to be examined for that organ and male (M) and female (F) groups.

Negative numbers indicate number of additional sections that should have been examined to satisfy protocol specifications.

Positive numbers indicate number of sections examined above that required by protocol.

0 indicates protocol specifications fulfilled.