MEMORANDUM

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

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

DATE: September 11, 1978

FROM : Thomas F.X. Collins, Ph.D. Thomas F.X. Collins
Whole Animal Toxicology Branch, HFF-155

SUBJECT: Aspartame (SC-18862). Review of four studies submitted by G.D. Searle as entries to Food Additive Master File 134, in response to your memo of September 1, 1978.

1. E-79. SC-18862: Segment II, an evaluation of the teratogenic potential in the rabbit. Final report. Hazleton Laboratories, Inc., Vienna, Virginia, October 8, 1974.

Procedure. New Zealand white rabbits were given a 5% or 15% aqueous suspension of aspartame in 1% aqueous Tween-80 by oral intubation twice per day from day 6 through day 18 of gestation. The dosage given was 0.75 g/kg/day for the low dose and 2.0 g/kg/day for the high dose. The control group received a 1% aqueous solution of Tween-80 by oral intubation twice daily during the same days of gestation in a volume equal to that received by the highest dose level. The study was done in three replicates (sub-groups A, B, C). The number of animals per subgroup ranged from 10 to 12 for the controls, from 11 to 14 for the low level, and 28 to 38 for the high level. Control females in each group were pair fed on a g/kg basis with the appropriate number of group-3 does from the corresponding subgroup which consumed the least amount of the diet on the preceding day (same day of gestation). To accomplish pair feeding, the control females in each subgroup were inseminated 24 hours following insemination of animals assigned the the corresponding high level group. Pair feeding began on day 6 and continued until sacrifice at day 29. During treatment, the animals were fasted 8-9 hours (1.5-2 hours preceding the first daily dose and ending after completion of the second dose).

Results. Mortality rate was slightly increased at both dose levels but this was not related to dose. Conception rates showed a dose-related decrease. Both experimental groups gained weight during the period of treatment while the controls actually lost weight. The pre-treatment food consumption rates were similar for the control and treatment groups. During treatment, the treated groups consumed significantly more food than the controls. Following treatment, the controls consumed slightly more food than during the treatment period, while the treated groups consumed slightly less, but still the treated groups consumed significantly more food than the controls.

There appeared to be a slight increase in pre-implantation loss at the high dose level. There appeared to be a decrease in the mean number

of live fetuses and an increase in the mean number of resorption sites at the high dose level. Statistics were only done on the combined data. If statistics were done on the subgroups, significant effects on these parameters might be noted. There was no increase in the number of does with implantation scars, resorption sites, or dead fetuses.

Mean fetal weight was significantly increased at the low dose level (0.75 g/kg) but was not affected at the high level. There appeared to be no effect on fetal length.

At the high dose level, there was an increase in the number of fetuses with abnormalities and an increase in the number of litters affected. No abnormalities were observed in the control animals (19 litters). At the low dose level, 1 of 198 fetuses (24 litters) showed gastroschisis with associated rotation of hindlimbs, rotation of the eye, and other anomalies. At the high dose level, 7 of 343 fetuses (6 litters out of 45) showed major and minor anomalies consisting in part of: bi-clefted lip, cleft palate, fused mandible, short maxillary bones, fusion and misalignment of caudal vertebrae, reduced ossification of thoracic and caudal vertebrae and phalanges, hydrocephalus, missing ribs, and ectrodactyly.

Problems. 1. The exact mechanism of the pair-feeding schedule is not clear.
2. Eighteen fetuses at the high level were too small to be processed, hence the evaluation of these fetuses is not available.

Conclusion. There were deleterious effects at the high dose level, 2.0 g/kg, but no effects at the low level, 0.75 g/kg. Rigorous pair feeding was certainly a factor.

2. E-83. SC-18862 Placebo: An evaluation of embryotoxic and teratogenic potential of specially prepared pelleted diet in the rabbit. Searle Laboratories, October 1974.

Procedure. This study was done to test the diet used in a previous segment II rabbit study conducted at Searle and Hazleton Laboratories. A commercially available diet from the same manufacturer served as the control. Sixty New Zealand white female rabbits approximately 9 months old were given one of the following diets: commercial control (10 animals), Searle control (25 animals), and Hazleton control (25 animals). The animals had a mean body weight of 3.8 kg. Searle and Hazleton diets were offered ad libitum to the appropriate groups of animals from the morning of gestation day 6 to the morning of gestation day 19. Commercial diet was given prior to and subsequent to this period. The animals were sacrificed near term (day 28) and the uterine contents examined. The diets were analyzed for moisture content, aflatoxins, aerobic bacteria, yeasts, and molds.

Results. Hazleton and Searle diets showed less moisture due to additional drying times these diets had prior to the pelleting process. There was also an increase in the amount of detectable yeasts and molds in the Searle and Hazleton diets.

Maternal survival, fertility, body weight, and food consumption were comparable among the three groups. In fact, animals on Searle and Hazleton diets gained slightly more weight than those on the commercial control diets. There was a decrease in mean litter size between the commercial diet and both the Searle and Hazleton control diets, as well as an increase in resorptions. The authors of the report state that an unusually large litter size was found in the commercial control diet, and that the mean litter size in the special diets is similar to that seen in historical rabbit data. The number of pregnant females with resorption sites and the mean number of resorption sites per litter were increased in the special diet groups. Weight and crown-rump lengths in both sexes for both special diets were increased over the commercial control diet.

Two gross abnormalities appeared in the Hazleton diet animals, one exencephaly and one cleft palate. No gross abnormalities were noted in the other two diets. One minor gross malformation was noted in each of the commercial control and Searle control diets; no minor malformations were noted in the Hazleton control diet. The total incidence of fetuses with major malformations observed during external, soft-tissue, and skeletal examination was: 0 of 64 fetuses in the commercial control diet (7 litters), 2 of 120 fetuses in the Searle control diet (2 of 19 litters), and 12 of 151 fetuses in the Hazleton control diet (5 of 20 litters). Soft-tissue examination revealed no additional fetuses with minor malformations. The incidence of fetuses with minor malformations detected during skeletal examination are: 0 of 34 fetuses in the commercial control diet (7 litters), 4 of 62 fetuses in the Searle control diet (3 of 19 litters), and 6 of 79 fetuses in the Hazleton control diet (4 of 20 litters).

In the commercial control diet group, the sole malformation observed was one fetus with a short tail. In the Searle control diet, there were two fetuses with bilateral folding of the retina, 4 fetuses with fused 4th and 5th sternebrae, and one fetus with bilateral flexure of the fore- and hind-limbs. In the Hazleton control diet, the skeletal anomalies seen were fused sternebrae (5) and poorly ossified skull bones (1). Other anomalies seen in the Hazleton animals were bilateral folding of the retina (7), perforation of the interventricular septum (1), agenesis of the kidney and ureter (1), hydrocephalus (3), and cleft palate (1).

Problems. 1. The age of the diets is unknown.
2. The exact treatment of the diets at the two laboratories is unknown.
3. There was a small number of control animals.

Conclusion. There was a higher number of abnormalities produced with the Searle and Hazleton diets than there was with the commercial control diet. The greatest number of abnormalities was produced with the Hazleton diet.

3. E-89. SC-18862: An evaluation of embryotoxic and teratogenic potential in the mouse. Searle Laboratories, July 1975.

Procedure. A Segment-II study was done on dietary aspartame using Charles River CD-1 albino mice 65-75 days old. Thirty-six females were assigned to each of 4 groups with an intended daily dose level of 0, 1.0, 2.0, or 4.0 g/kg, but the animals actually received approximately 40% more than the intended dose (1.4, 2.7, and 5.7 g/kg, respectively). The compound was given on days 6-15 of gestation. The animals were sacrificed on day 18.

Results. All females survived to day 18. There was a slight decrease in conception rate at the 4.0 g/kg level, but this was not significant. There was no compound-related effect on food consumption. There was no effect on the number of totally resorbed litters, mean litter size, number of resorption sites per litter, mean fetal body weight, or mean fetal length.

The incidence of major malformations was the following: 1 of 250 fetuses in the control group (1 of 25 litters), 0 of 251 fetuses of the low dose group (24 litters), 1 of 261 fetuses of the medium dose group (1 of 25 litters), and 0 of 204 fetuses of the high dose group (20 litters). The control fetus with abnormalities had a hypoplastic 4th thoracic vertebral centrum. The fetus from the medium dose group had exencephaly, cleft palate, and bilaterally open eyes. Skeletal variants were noted, but there was no statistically significant difference which could be related to dosage.

Conclusion. No compound-related teratogenic effects were detected in mice at dose levels up to 4.0 g/kg.

4. E-90. SC-18862: An evaluation of embryotoxic and teratogenic potential in the rabbit. Searle Laboratories, July 1975.

Procedure. New Zealand white female rabbits approximately 8 months of age were randomly distributed among the following groups: 0, 0.5, 1.0, or 2.0 g/kg aspartame, or 0.82 g/kg L-phenylalanine (L-phen), or 1.10 g/kg L-aspartic acid (L-asp). The compound and/or vehicle was intubated twice per day in 2 doses, separated by at least 3 hours, on days 6-18 of gestation. The compounds were suspended in a solution of 0.5% carboxymethyl cellulose and 1% Tween-80. L-phen and L-asp, dietary amino acids, are the principal constituents of aspartame. Food consumption was recorded daily during gestation. Body weights were recorded on days 0, 3, 6, 10, 13, 15, 18, 22, and 28. Cesarean sections were done on day 28, and standard teratological observations were made. Approximately 50 rabbits per dose were used.

Results. Survival rate of the dams was not affected by aspartame or the 2 dietary amino acids. Conception rate was slightly decreased at the high

dose level, and the proportion of pregnancies which terminated in abortion was significantly greater than the control group. From day 13 to day 28, the average body weight of the females given the high dose level was significantly less than the weight of the controls.

Mean food consumption for control, 0.5, and 1.0 g/kg aspartame decreased to 75-90% of the daily pretreatment values. Daily food consumption in the high dose group decreased to 25-35% of pretreatment values and was significantly less than the control values. After treatment, i.e., by day 22 of gestation, food consumption in the high dose group was back to normal. Food consumption in the L-phen females decreased to 50-60% of the pretreatment mean and significantly less than the control animals on each day of treatment. Food consumption returned to normal on day 20.

A decrease in the number of litters having completely viable fetuses was noted at the high level aspartame and with L-phen. The number of litters completely resorbed and the number of resorption sites per litter were increased at the high dose level of aspartame. Mean litter size was not affected by aspartame dosage. Fetal body weight and crown-rump lengths were significantly decreased in both sexes at 2.0 g/kg aspartame and L-phen.

The number of litters containing pups with grossly visible abnormalities was increased in the 2.0 g/kg aspartame and L-phen groups. Cleft palate appeared to be significantly increased at the high dose level of aspartame. There was a significant increase in the number of rabbits with an extra pair of ribs, as well as a significant decrease in ossification of the second sternebral center, increased absence of the 6th sternebral center, increase in unossified metacarpals, and an increase in unossified tarsals.

Conclusion. There were deleterious affects at the high dose level of aspartame, 2.0 g/kg, in rabbits. Dosage up to 1.0 g/kg/day did not appear to affect pregnant rabbits.

General conclusions. 1. Aspartame appeared to be non-teratogenic in the mouse feeding study at dose levels of 1.4, 2.7, and 5.7 g/kg.

2. In both rabbit studies, aspartame appeared to cause birth defects at the high level (2 g/kg).

3. In the comparison of diets used in rabbit studies at Searle and Harleton, more abnormalities were seen in the special diets than in the control diets but the treatment and the age of the diets are not given.

MASTER FILE ENTRY	TITLE/AUTHORS	ABSTRACT/REASON FOR STUDY	DATE SI TO U.S
E-11	Two Generation Reproduction Study Rats P-T 867H71 Author: Hazelton Laboratories	To evaluate and characterize effects of SC-18862 on the reproductive performance of albino rats. Dietary administration carried on through 2 parental generations and two one-litter filial generations.	10/
E-12	SC-18862: Mutagenic Study in Rats P-T 869H 70 Final Report Author: Hazelton Laboratories	The purpose of this study was to determine the potential mutagenic effect of test material SC-18862 on the bone marrow and spermatogonial cells of the rat.	10/
E-13	SC-19192: Segment III Perinatal Weaning Study in the Rat P-T 1011H72 Final Report Author: Hazleton Laboratories	This study was conducted to evaluate the potential effects of SC-19192 on the perinatal and postnatal phases of the reproductive process in albino rats, with emphasis on evaluation of parturition, neonatal viability, and growth of the newborn.	10/
E-14	SC-18862: Behavioral Effects of chronic Feeding of L-phenylalanine and SC-18862 to Weaning Rats Biology Document No. 793 Author: W.J. Potts	In an effort to compare APM with phenylalanine, and employing 5% L-phenylalanine diet in rats as the model, a 13 week experiment was conducted in weaning rats. In this behavioral toxicity study, dose levels of APM were chosen so as to provide an amount of phenylalanine equivalent to 2.5% and 5.0% in the diet.	10/
E-15	SC-18862: Metabolism of Aspartame-Volume I Parts I-XIV Author: Dr. R.E. Ranney, et al.	Studies of the pharmacokinetics and metabolism of SC-18862 have been carried out in rats, mice, dogs, rabbits, rhesus monkeys and man.	10/
E-16	Sweetening Agent Bibliography		10/1
E-17	SC-18862: The Metabolism of Aspartame Volume II Parts XV - XIX Author: Dr. R.E. Ranney, et al	See E-15	11/3

MASTER FILE ENTRY	TITLE/AUTHORS	ABSTRACT/REASON FOR STUDY	DATE TO U.
E-5	Evaluation of Embryotoxic and Teratogenic Potential in the Rat P-T 851S70 Authors: R.E. Schroeder and R.G. McConnell	Evaluate embryotoxic and/or teratogenic potential of SC-18862 when administered orally in the diet to the albino rat. This study design is commonly referred to as Segment II of the Teratology-Reproduction profile.	8/
E-6	SC-19192: Two Week Oral Toxicity Study in the Mouse P-T 885S70 Authors: K.S. Rao, T.B. Martinez, R.D. Hemm and R.G. McConnell	The finished product of SC-18862 may contain 0-1% of a degradation product, SC-19192. Preclinical testing of SC-19192 for its potential toxicity was performed.	8/
E-7	SC-19192: Two Week Oral Toxicity Study in the Rat. P-T884S70 Authors: K.S. Rao, J. Mauro and R.G. McConnell	Same as above.	8/1
E-8	SC-19192: Five Week Oral Toxicity Study in the Rat P-T972S71 Authors: K.S. Rao, C. Staunton, R.G. McConnell	SC-19192 administered to young albino rats of both sexes for five consecutive weeks to evaluate safety of multiples of the model estimated daily human dosage and to induce and define adverse effects as might occur only at prodigous multiples of such dosages.	8/1
E-9	Toxicological Evaluation in the Neonatal Rat P-T 893H71 Hazelton Laboratories Report	To evaluate and characterize the effects of SC-18862 on hematological and biochemical parameters and on tissues of rats one through 21 days.	10/
E-10	Toxicological Evaluation of SC-18862: Evaluation of Reproductive Performance P-T 857S70 Authors: R.E. Schroeder, K.S. Rao, and R.G. McConnell	To evaluate effects of SC-18862 to the male and female albino rat prior to mating and to the pregnant female during the entire period of gestation and lactation. (Segment I teratology)	10/1

MASTER FILE ENTRY	TITLE/AUTHORS	ABSTRACT/REASON FOR STUDY	DATE TO U.
1-D	Analytical Data and Specifications of Food Grade Aspartame Authors: Dr. E. Lau, Dr. G. Anthony J. Damascus, B. Smith	Analytical data of aspartame, specifications for food grade aspartame and its directions for testing	11
2-D	Analytical Methods for Aspartame and DKP in Processed Food	Document in General Foods Master File # 135	1/
E-1	A Sweetening Agent Pharmacological Studies Author: Donald L. Cook, Ph.D	SC-18862 was subjected to a wide variety of pharmacological tests in order to delineate any possible adverse effects of the compound on the gastrointestinal system, cardiovascular system or central nervous system	8/
E-2	SC-18862: Four Week Oral Tolerance Study in the Mouse P-T No. 815S69 Authors: K.S. Rao, T.B. Martinez and R.G. McConnell	SC-18862 was administered orally in the diet to 8 week old mice of both sexes for four consecutive weeks to establish a desirable dose range and maximum tolerated dose for subsequent toxicity studies of longer duration	8/1
E-3	SC-18862: Four Week Oral Tolerance Study in the Rat P-T 814S69 Authors: K.S. Rao, T.B. Martinez and R.G. McConnell	SC-18862 was administered orally in the diet to 8 week old albino rats of both sexes for four consecutive weeks to establish a desirable dose range and maximum tolerated dose for subsequent toxicity studies of longer duration.	8/1
E-4	SC-18862: Nine Week Oral Toxicity Study in the Rat. Authors: R.D. Hemm, K.S. Rao, T.B. Martinez, D.W. Calhoun and J.E. Mayer P-T847S70	To establish a desirable dose range for subsequent behavioral and toxicity studies of longer duration, and to provide preliminary information on the effects of 5% L-phenylalanine or 9% SC-18862 diet on body weight gain, food intake and physical examination, clinical laboratory and postmortem findings after nine weeks of compound administration.	8/1