SC-18862: 52 WEEK ORAL TOXICITY STUDY IN THE INFANT MONKEY

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INTRODUCTION

In this toxicity study SC-18862, a nutritive artificial sweetening agent, was administered orally in the milk formula to infant Rhesus monkeys for 52 consecutive weeks. SC-18862 is a dipeptide and is split to its constituent moieties by peptidases in the digestive tract.

This study was designed to determine the adverse effects, if any, of SC-18862 ingestion on the neonatal Rhesus monkey, and also whether all such effects were identical in nature and magnitude to those produced by an equimolar quantity of L-phenylalanine.[1]

A research project involving repeated daily oral administration of any agent to a sizable population of baby monkeys, commencing at birth and continuing uninterrupted throughout the 1st year of life, is a major undertaking fraught with hazard, even for the partially initiated.

Thus, this study was performed at the Primate Research Center, Madison, Wisconsin under the direction of the late Dr. Harry A. Waisman, Prof. of Pediatrics and Director, Joseph P. Kennedy Memorial Laboratories. His established expertise in research involving phenylalanine and the neonatal

* deceased

Rhesus monkey was invaluable, and his unfortunate demise necessitated revision of the initial objectives of this study. This report does provide valuable physical examination and clinical laboratory data enabling comparison of SC-18862 with known effects of L-phenylalanine.

METHODS

Material evaluated.

SC-18862 is a fine white powder with the chemical name L-aspartyl, L-phenylalanine methyl ester. Three lots (74020, 75060B, 74060) were used throughout this study. These lots contained from 0.2 to 1% of SC-19192 (Diketopiperazine; DKP), a conversion product of SC-18862.

Animals, housing and diet.

Infant Rhesus monkeys (Macaca mulatta) from full-term, normal pregnancies were separated from their mothers within 6 hours after birth and transferred to individual heated cages.

During the first 24 hours of life, the infants were fed a 10% glucose solution at four-hour intervals; during the second day, this diet was supplemented with equal volumes of a commercial milk preparation (Similac, Ross Laboratories, Columbus, Ohio; Control diet, CD). Thereafter, the infants were fed CD ad libitum at four hour intervals until they were placed on the experimental liquid formula.

During the training period, the infant was gently wrapped in a cloth diaper and held while fed from a toy nursing bottle and nipple. Four feedings per day was the preferred number for this experiment. Later,

between days 12 and 30, the animals were weaned and fed from a small cup; on or after day 31 they were fed from a large cup.

Compound administration.

Similac formula was supplemented with SC-18862 on a "phenylalanine equivalent" basis: 1.83 g L-aspartyl, L-phenylalanine methyl ester contains 1.0 g L-phenylalanine. The SC-18862 concentration was incrementally increased, based on acceptance by the infant.

Age	Code	Aspartyl Phenylalanine	= L-Phenylalanine
Day 3- Day 9	1/8th	.0029 g/cc	.0016 g/cc
10- 19	1/4	.0057 g/cc	.0031 g/cc
20- 29	3/8	.0086 g/cc	.0046 g/cc
30- 119	1/2	.0114 g/cc	.0063 g/cc
120- 179	5/8	.0143 g/cc	.0078 g/cc
180 229	3/4	.0171 g/cc	.0094 g/cc
230 269	7/8	.02 g/cc	.011 g/cc
270 365	1	.022 g/cc	.012 g/cc

Milk intake was carefully recorded for each feeding, so that the amount of SC-18862 consumed per day per kg of body weight could be calculated, allowance being made for spillage. When the animals were 3 months old, a quarter of an apple and a quarter of an orange were placed in the cage once a day. The infant monkeys were fed SC-18862 with the milk formula. Water was available ad libitum. Animal quarters were air-conditioned with thermostats set to maintain a room temperature of 72°F; artificial fluorescent lighting was provided on a 14 hour daily photoperiod.

Experimental design.

Seven newborn Rhesus monkeys, five males (M34, M38, M64, M79, P53) and two females (N14, P60), were randomly divided into three groups.

Treatment Group	Intended Dosage g/kg/day	Multiple of Estimated Daily Human Intake*	Animal No.	Sex	Date of Birth	Start Supplement Age (Days)
Low	1	33	P53	M	8-28-70	6
			P60	F	9- 6-70	3
Medium	3	100	M64	M	3-19-70	3
			M79	M	4- 5-70	3
			N14	F	4-26-70	2
High	4→6	133→200	M34	M	1- 5-70	9
			M38	M	1-13-70	1

* Based on 30 mg/kg oral intake daily to a 27 kg child.

The treatment was arbitrarily terminated by the late Dr. Waisman's staff as indicated below.

Treatment Group	Animal No.	Treatment Initiated	Treatment Terminated	Total Days on Treatment
Low	P53	9- 3-70	3-31-71	210
	P60	9- 9-70	3-31-71	204
Medium	M64	3-23-70	3-18-71	360
	M79	4- 8-70	4- 4-71	362
	N14	4-28-70	4-25-71	363
High	M34	1-14-70	1- 5-71	357
	M38	1-14-70	10-20-70	279

Physical examinations and observations.

Animals were observed daily at the time of dosing and intermittently between dosing periods for survival and behavioral changes. Body weights were recorded each day in the morning. Head circumference and body length (crown to heel length) were recorded at 4 week intervals. An evaluation of

general motor and behavioral activity, locomotion, external appearance of teeth, nose, eyes, ears, perineum, hair coat and digital palpation for tissue masses was conducted immediately prior to the initiation of compound administration, and subsequently concurrent with each body weight measurement. Unusual signs, including indications of systemic pharmacologic or toxicologic effects, were routinely recorded at this time and whenever warranted.

Clinical laboratory procedures.

Hematologic and clinical chemical examinations which were performed on blood specimens of all animals, were collected via the saphenous vein at 3, 6, 9 and 12 months of compound administration.

Hematology. The following hematologic parameters were measured:

Parameter	Method
Hematocrit (micro)	Micro method[2]
Hemoglobin	Cyanmethemoglobin[3]
Total RBC count	Coulter Counter[4]
Total WBC count	Coulter Counter[4]
Diff. WBC count	Smear[5]

Clinical chemistry. The following (plasma chemistry) parameters were measured for all groups:

Parameter	Method
Blood (plasma) urea nitrogen	Urograph method[6]
Uric acid	Brown[7]
Glutamic oxalacetic transaminase	Reitman & Frankel[8]
Alkaline phosphatase	Klein et al.[9]
Bilirubin	Malloy & Evelyn[10,11]

Parameter (cont.)	Method (cont.)
Glucose	Nelson & Somogyi[12,13]
Calcium	Barr[14]
Inorganic phosphate	Fiske & Subbahow[15]
Cholesterol	Abell et al.[16]
Total protein	TS Meter[17]
Phenylalanine	Undenfriend & Cooper[18]
Tyrosine	La Du & Michael[19]

Serum phenylalanine and tyrosine were monitored twice a week for the first 13 weeks, weekly for the next 17 weeks and once every two weeks thereafter.

Urinalysis. Spontaneously voided urine specimens from individually housed monkeys were collected at 3, 6, 9 and 12 months of treatment. The following parameters were measured.

Parameter	Method
Specific gravity	Total solids meter
pH	Labstix (Ames)
Occult blood	Labstix (Ames)
Protein	Labstix (Ames)
Glucose	Labstix (Ames)
Ketones	Labstix (Ames)
Bilirubin	Labstix (Ames)
Phenylketones	Phenistix (Ames)

RESULTS

ANTEMORTEM OBSERVATIONS

The availability of acceptable historical and contemporary data on untreated control monkeys from the Waisman group reduced the necessity of a concurrent control group. The extremely limited availability of newborn Rhesus, as well as limitations in adequately skilled laboratory personnel, likewise contributed to our decision to eliminate the requirement of a concurrent control group in this study.

For comparative purposes the normal range of values from 14 historical control monkeys is superimposed on Figures 1-9.

Compound consumption.

The treatment of monkeys with SC-18862 was initiated on the basis of availability of newborn monkeys as indicated on page 4. The sudden demise of Dr. Waisman necessitated termination of the study. At that point in time, the medium and high dose monkeys had completed 52 weeks of treatment, and the low dose monkeys had completed 29-30 weeks of treatment.

Mean values for SC-18862 ingestion by the low and medium dose group animals over the treatment period (Table 1) were within 5% of the proposed doses of 1.0 and 3.0 g/kg. The intended dosage of SC-18862 for the high dose group was 4 to 6 g/kg; because of an unanticipated decrease in the intake of milk formula, presumably due to the intense sweetness of SC-18862, the realized mean intake of SC-18862 over the entire study was 3.6 g/kg (range 1.21 to 5.33 g/kg). Hence, the SC-18862 intake of high dose group animals was not notably different from the medium dose group animals. Irrespective of the actual intake of SC-18862 levels, the results of this study are presented as

Table 1

SC-18862: 52 WEEK ORAL TOXICITY STUDY IN THE INFANT MONKEY

Consumption of SC-18862 (gm per kg per day)

(Mean Values)

Treatment Group	Treatment Intervals (days) 0-9	10-19	20-29	30-39	40-49	50-59	60-69	70-79	80-89	90-99	100-109	110-119	120-129
ow Dose	0.77	0.95	0.97	1.09	0.98	0.99	1.00	0.99	0.98	0.94	0.91	0.98	0.97
edium Dose	0.94	1.76	2.90	3.55	3.51	3.54	3.20	3.25	3.42	3.23	3.35	3.24	3.85
igh Dose	1.21	1.82	1.83	2.36	3.21	3.68	3.37	3.54	3.67	3.11	3.20	3.22	3.24

	Treatment Intervals (days) 130-139	140-149	150-159	160-169	170-179	180-189	190-199	200-209	210-219	220-229	230-239	240-249
ow Dose	1.06	1.29	1.36	1.13	1.09	0.99	0.93	0.62	-	-	-	-
edium Dose	3.69	3.73	3.44	3.49	2.78	2.80	2.75	2.54	2.36	2.38	2.66	2.48
igh Dose	3.88	4.38	4.38	4.38	4.33	5.33	4.69	3.83	3.99	4.21	4.84	4.24

	Treatment Intervals (days) 250-259	260-269	270-279	280-289	290-299	300-309	310-319	320-329	330-339	340-349	350-359	360-365	Mean 0-369
w Dose	-	-	-	-	-	-	-	-	-	-	-	-	0.97*
edium Dose	2.37	2.04	2.19	2.43	2.94	2.77	3.05	3.65	3.19	3.02	3.07	2.88	3.01
igh Dose	3.70	3.58	3.75	4.32	4.83	4.33	3.30	4.04	3.37	3.11	3.31	2.50	3.62

*Mean for 0-209 days.

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SC-18862: 52 WEEK ORAL TOXICITY STUDY IN THE INFANT MONKEY

Consumption of SC-19102 (mg per kg per day)

(Mean Values)

eatment Group	Treatment Intervals (days) 0-9	10-19	20-29	30-39	40-49	50-59	60-69	70-79	80-80	90-99	100-109	110-119	120-129
w Dose	3.84	4.75	4.85	5.45	4.90	4.95	5.01	4.97	4.90	4.71	4.54	4.92	4.87
dium Dose	4.71	8.80	14.48	17.73	17.56	17.71	16.02	16.25	17.11	16.17	16.74	16.22	19.26
gh Dose	6.03	9.08	9.15	11.82	16.07	18.42	16.84	17.68	18.37	15.54	16.01	16.08	16.21

	Treatment Intervals (days) 130-139	140-149	150-159	160-169	170-179	180-189	190-199	200-209	210-219	220-229	230-239	240-249
w Dose	5.29	6.46	6.82	5.67	5.44	4.93	4.67	3.10	1.54	-	-	-
dium Dose	18.45	18.67	17.20	17.47	13.88	13.99	13.76	12.72	11.82	11.88	13.31	12.42
gh Dose	19.41	21.88	21.92	21.90	21.63	26.67	23.46	19.14	19.93	21.03	24.18	21.12

	Treatment Intervals (days) 250-259	260-269	270-279	280-289	290-299	300-309	310-319	320-329	330-339	340-349	350-359	360-365	Mean 0-369
ow Dose	-	-	-	-	-	-	-	-	-	-	-	-	4.84
edium Dose	11.87	10.18	10.94	12.17	14.72	13.87	15.27	18.27	15.95	15.12	15.33	14.42	15.07
igh Dose	18.49	17.92	18.74	21.58	24.16	21.66	16.52	20.19	16.85	15.56	16.54	12.48	18.12

Mean for 0-209 days.

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data for the low dose group (0.97 g/kg intake), medium dose group (3.01 g/kg intake), and high dose group (3.62 g/kg intake), according to the original placement of animals within each group.

As pointed out in the methods section, the SC-18862 lots employed in this study contained 0.2 to 1% SC-19192, a conversion product of SC-18862. The actual group mean daily ingestion of SC-19192 (Table 2) was computed from the actual intake of SC-18862 and from analytical data (Quality Control Department, Searle Laboratories) indicating the SC-19192 content of each individual lot of SC-18862 employed in this study. The group mean intake of SC-19192 over the entire study was 4.84, 15.07 and 18.12 mg/kg/day for the low, medium and high dose groups, respectively.

Growth and food consumption.

Absolute body weight and weight gain (g/kg/day) of individual monkeys in each group are presented in Figures 1, 2 and 3. Body weight gain per ml milk formula consumed and actual intake of liquid diet over the 52 week treatment period are depicted in Figures 4, 5 and 6.

The body weight in kilograms was within normal limits for P60, M64 and M34. One high dose monkey, M38, and two medium dose monkeys, M14 and M79, showed slightly lower body weight, but there seemed to be a leveling off in the weight as the animals approached one year on the diet.

Low dose monkey P53 exhibited evidence of physical deficiencies, apparently congenital in origin, shortly after birth. The animal was examined by selected consultants, and its suitability for inclusion in the study was questioned. A precise account of their findings is not available. The animal was continued on study irrespectively, however, since the supply of baby Rhesus was very limited. Subsequent poor growth of this animal (Fig. 1) was due to inappetance and may reflect the initial difficulties.

Figure 1

SC-18862: 52 WEEK ORAL TOXICITY STUDY IN THE INFANT MONKEY

Body Weight and Weight Gain for the Low Dose Group

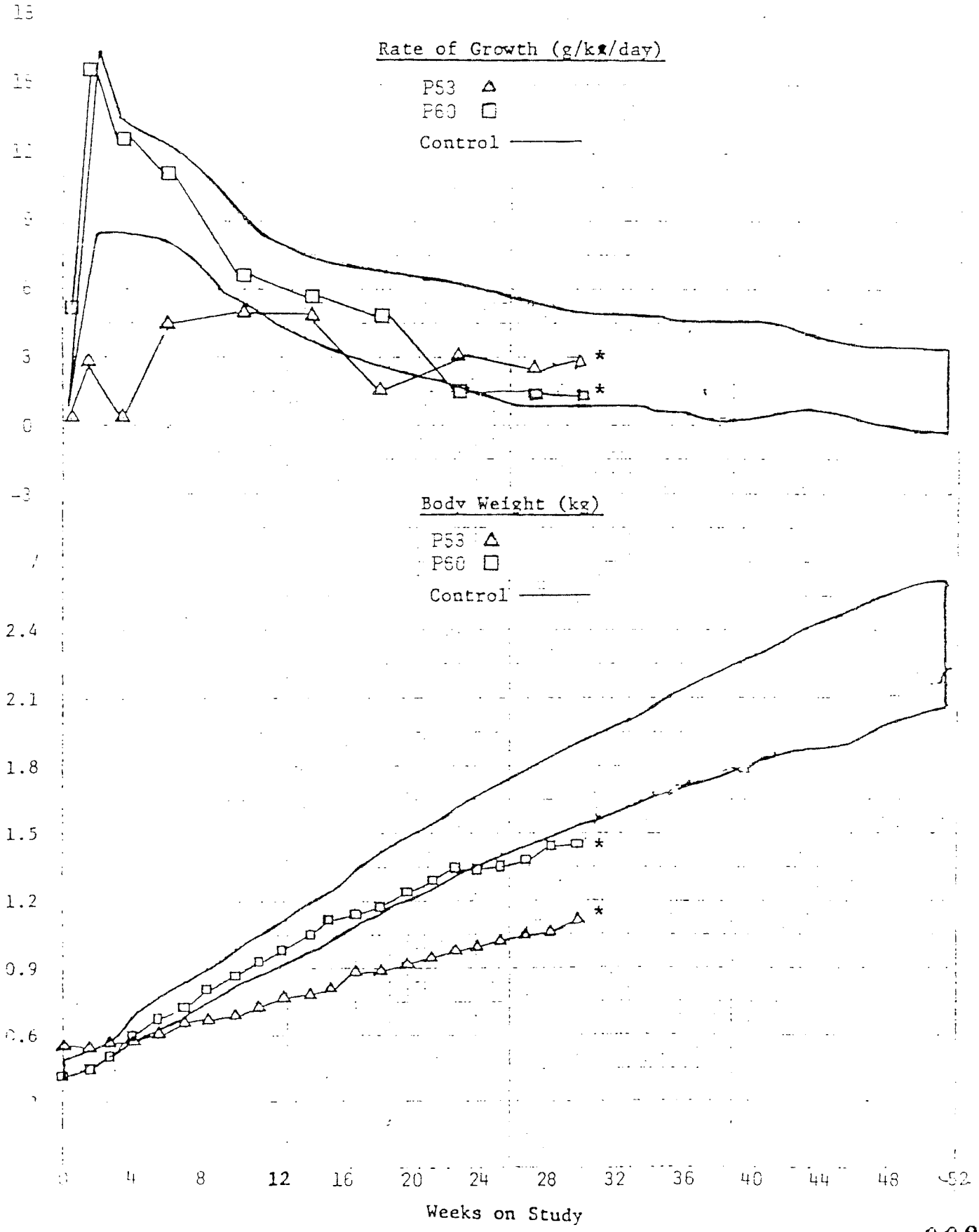

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Figure 2

SC-18862: 52 WEEK ORAL TOXICITY STUDY IN THE INFANT MONKEY

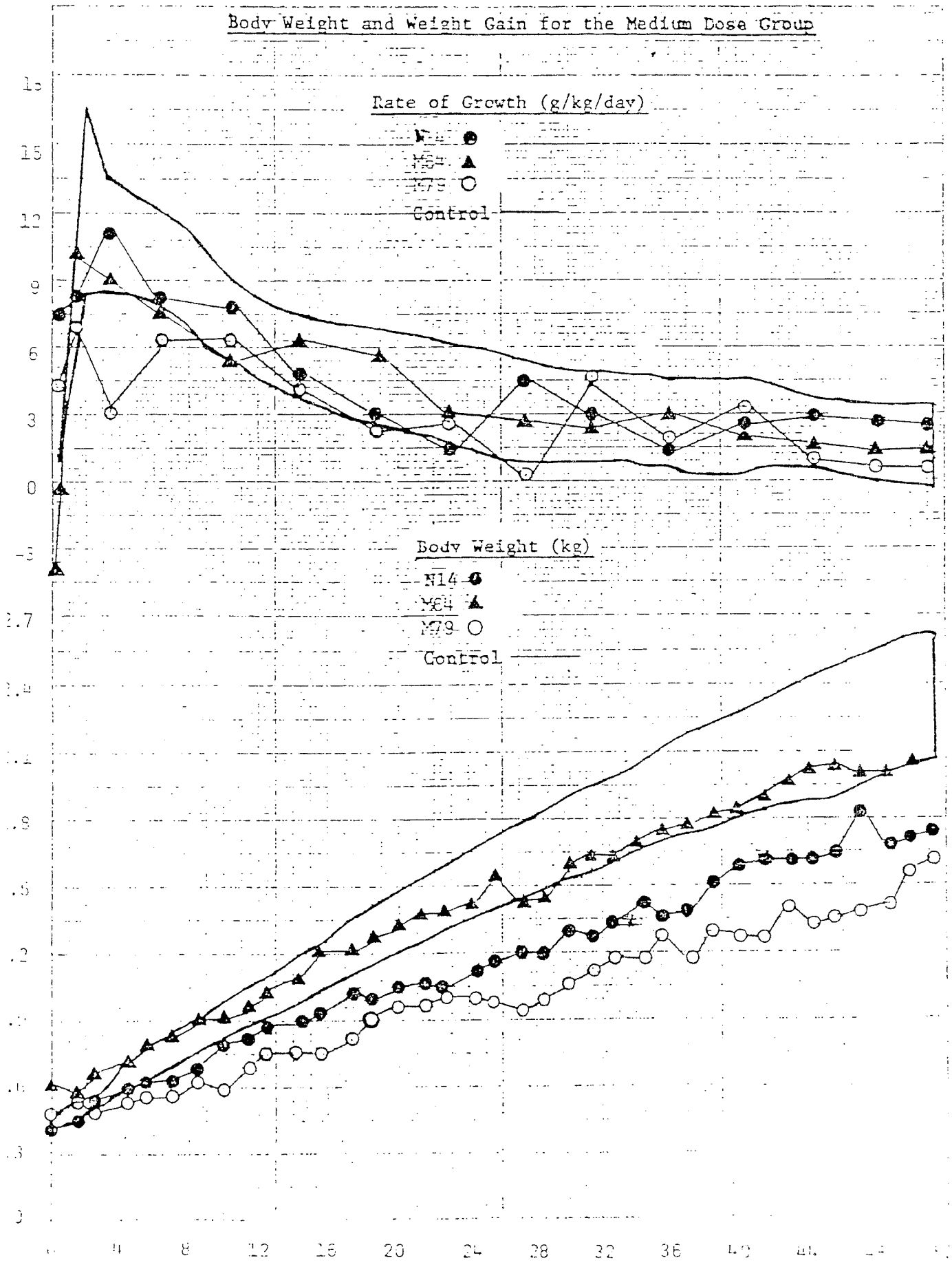

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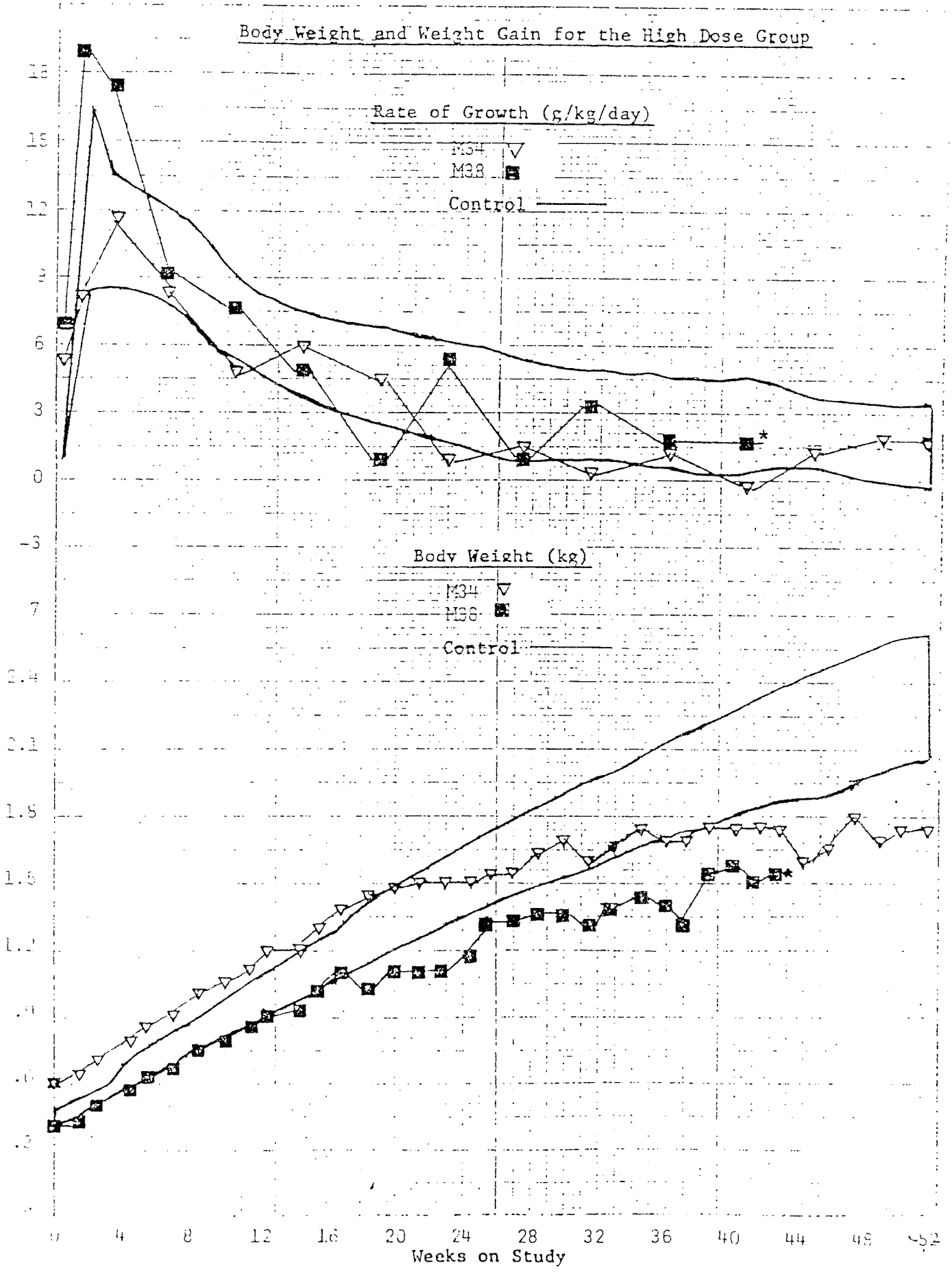
Figure 3

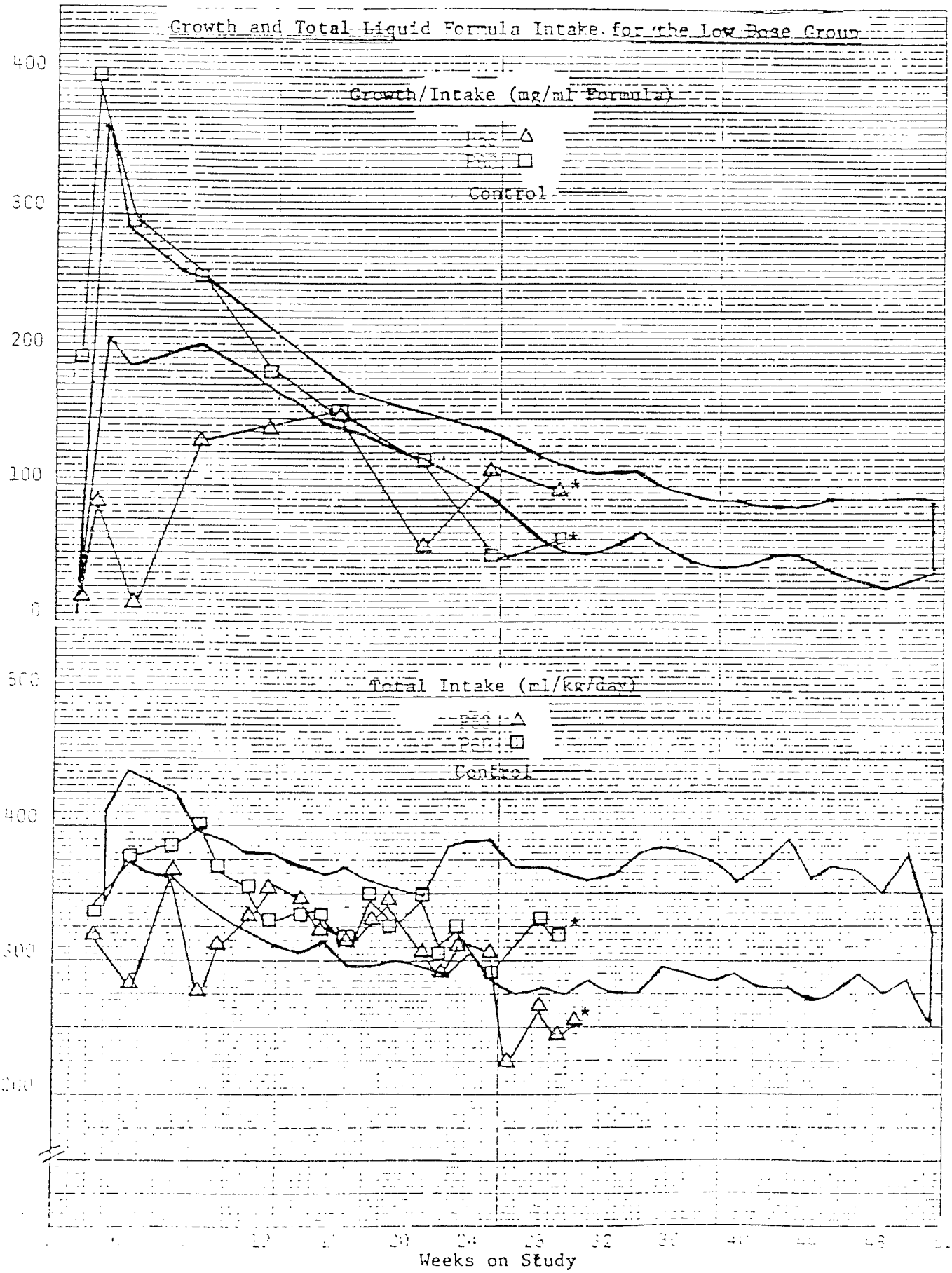
SC-18862: 52 WEEK ORAL TOXICITY STUDY IN THE INFANT MONKEY



* Died at week 43 of treatment.

Figure 4

SC-18862: 52 WEEK ORAL TOXICITY STUDY IN THE INFANT MONKEY



* Terminated the study.

Figure 5

SC-18862: 52 WEEK ORAL TOXICITY STUDY IN THE INFANT MONKEY

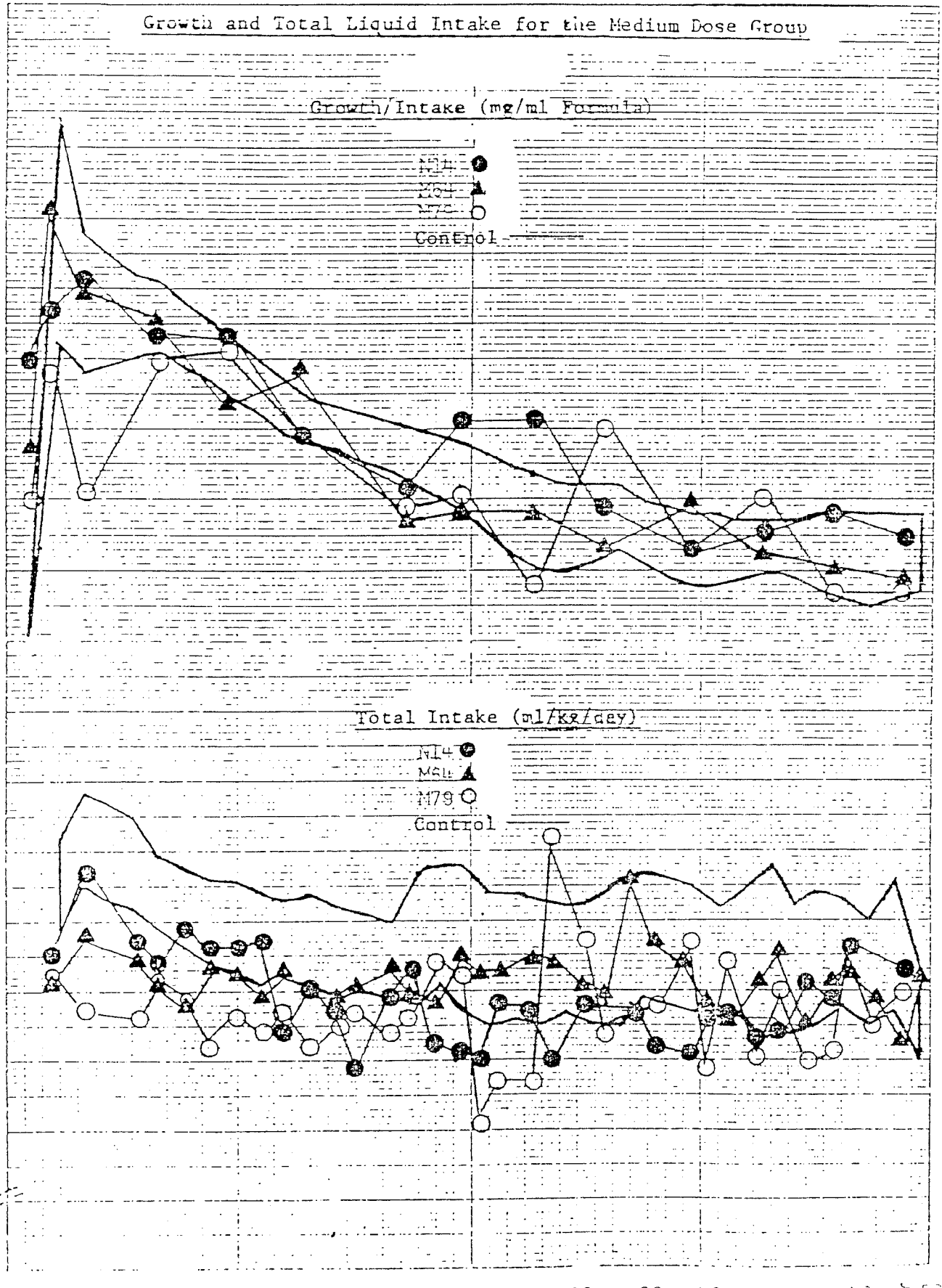


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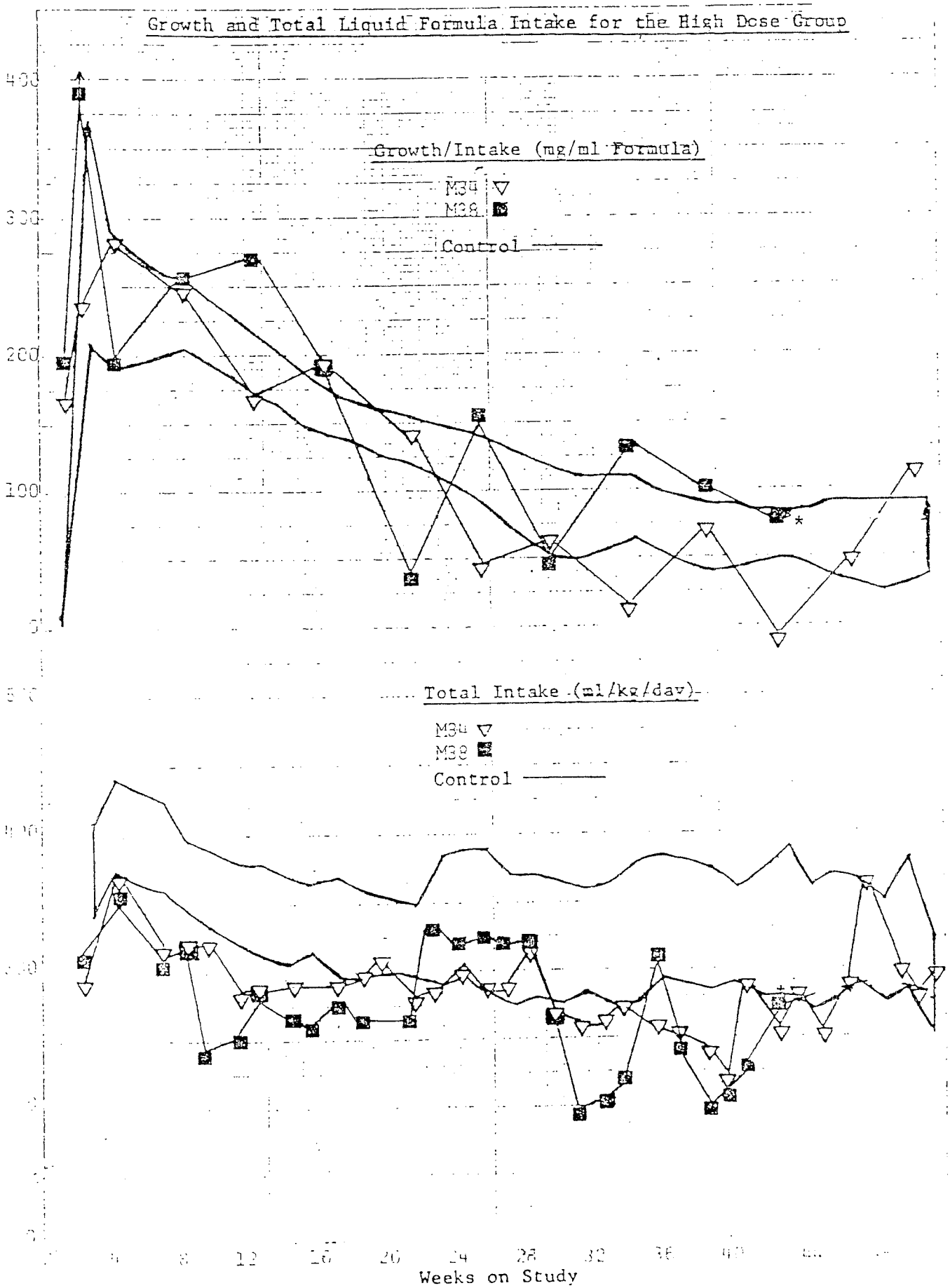
Figure 6

SC-18862: 52 WEEK ORAL TOXICITY STUDY IN THE INFANT MONKEY



* Died at week 43 of treatment.

Relative weight gain (g/kg/day) of all treated animals except monkey P53 was comparable to historical controls.

Rate of growth expressed per unit of diet intake (Figs. 4, 5, 6) was within normal limits despite the falling off of absolute body weight (Figs. 1, 2, 3). This indicates that the dipeptide was utilized efficiently and did not effect the efficiency of food conversion.

There was a marked decrease in total intake of milk formula in all the treated animals (Figs. 4, 5, 6). This could be attributed to the intense sweetness (200 x sucrose) of the dipeptide.

Individual daily body weight and milk formula intake of each experimental monkey may be found in the Appendix.

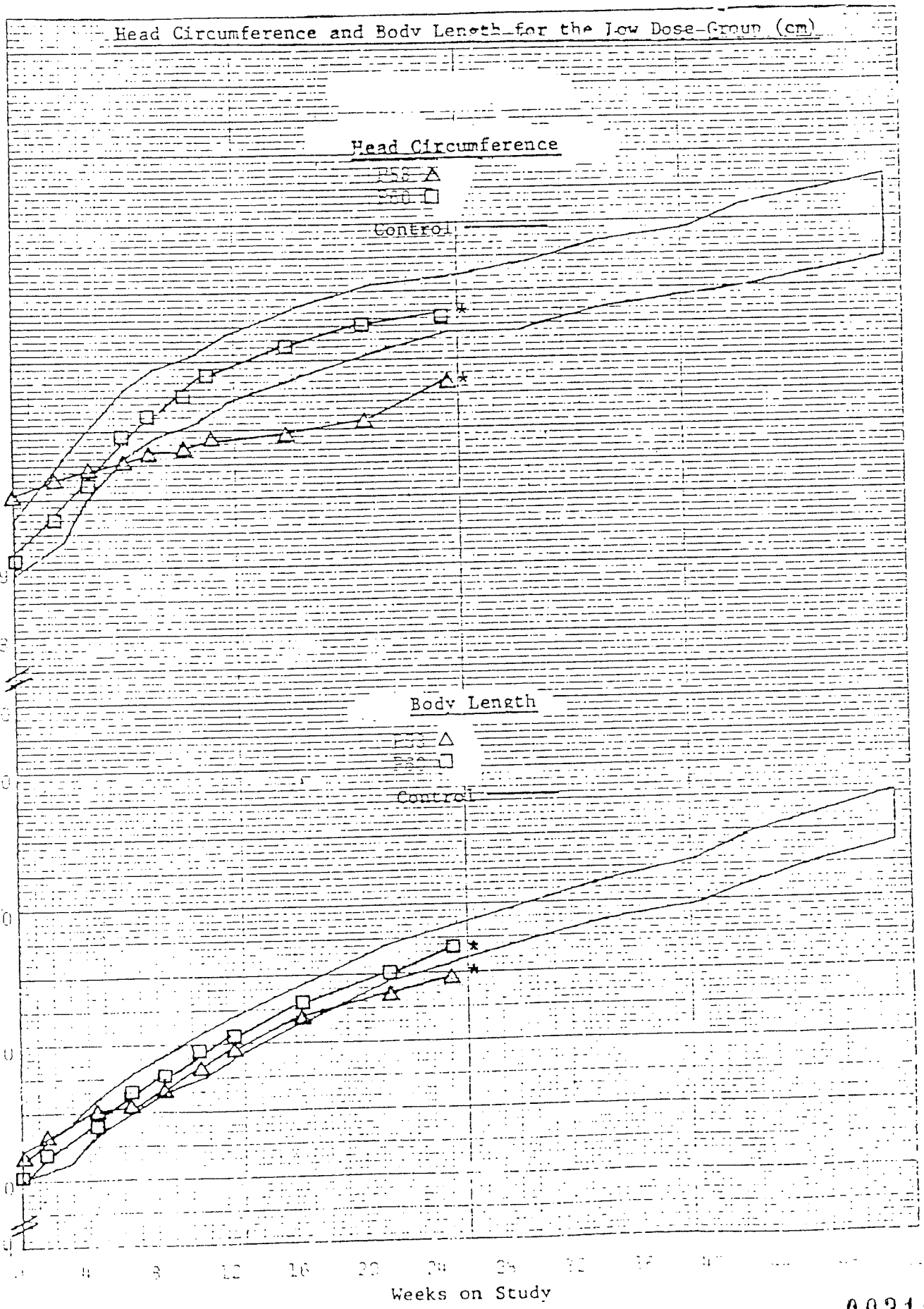
Body length of all treated animals is essentially within the historical control range; head circumference is likewise within historical control range for 1/2 low level, 1/3 medium level and 2/2 high level monkeys, but is below control level in the remaining animals (Figs. 7, 8, 9). The decrease in head circumference during treatment in low dose monkey P53 (Fig. 7) could be attributed to a proportional decrease in the relative weight gain (g/kg/day) of this monkey. Underdevelopment of this monkey is presumably related to the physical deficiencies observed at birth. An apparent decrease in the head circumference observed during treatment in two medium dose monkeys, M79 and N14 (Fig. 8), is attributed to a relatively lower head circumference at birth.

Observations, physical and behavioral signs.

All animals in the medium and high dosage groups exhibited seizure activity. Seizures were observed for the first time following 218 days of

Figure 7

SC-18862: 52 WEEK ORAL TOXICITY STUDY IN THE INFANT MONKEY



* Terminated the study.

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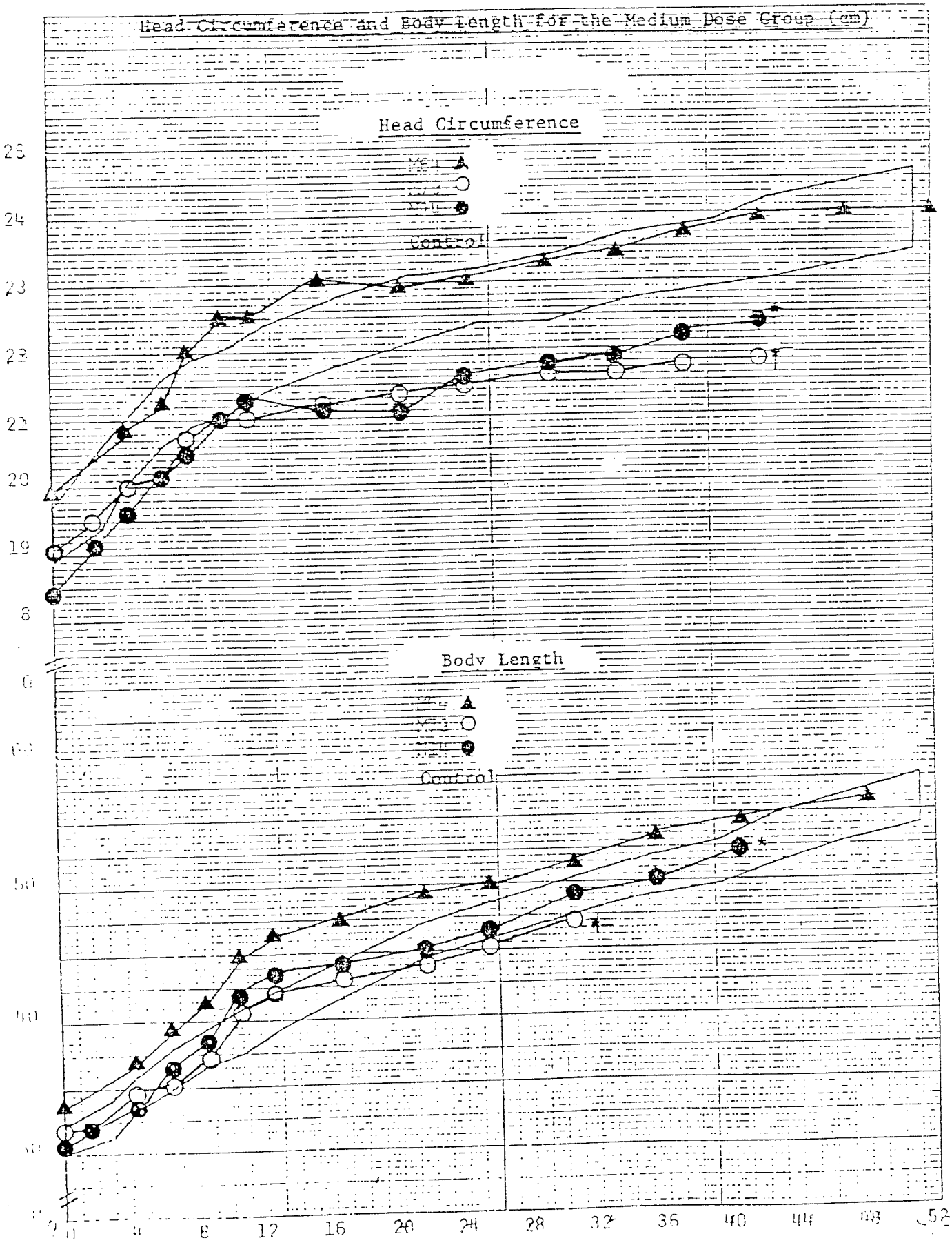
Figure 8

SC-18862: 52 WEEK ORAL TOXICITY STUDY IN THE INFANT MONKEY



* Data not available.