6394

G-000-307 .16

UNPUBLISHED SUBCHRONIC TOXICITY STUDY: NAPHTHALENE (C52904) FISCHER - 344 RATS - (USED AS A REFERENCE IN OU1 AND OU5 RI REPORTS)

03/04/80

C52904
NTP
75
REPORT

MAR 13 1980
RECEIVE

Battelle's Columbus Laboratories
Subcontract No. 76-34-106002
March 4, 1980

Subchronic Toxicity Study: Naphthalene (C52904), Fischer 344 Rats

Introduction

This subchronic gavage toxicity study was conducted to determine Maximum Tolerated Doses for the 104-week chronic toxicity study. Dose selections for this subchronic study were based on clinical and pathological results from a two-week repeated dose study, and were confirmed by Charles Rose, Tracor Jitco, Incorporated, in a letter dated April 20, 1979. In this repeated dose study, there was no mortality at 400 mg/kg, but clinical signs of lethargy and roughened haircoats, and weight gain depressions of 30-50 percent were recorded. There was marked lethality at 800 mg/kg. The dose selections were 400, 200, 100, 50, and 25 mg/kg for both sexes and were to be administered at the rate of 5 ml/kg of animal body weight.

Materials and Methods

C-9 Subchronic

Chemical

Naphthalene, manufactured by Fisher Scientific Company, was received from Midwest Research Institute (MRI) on October 10, 1978. The MRI Analytical Report indicated two impurities (0.13 and 0.02 percent of the major peak area) and a water content of 0.23 $\pm$ 0.04 percent. Naphthalene is a white crystalline solid which is relatively stable when stored at room temperature (m.p. 80-81 C). A Copy of the Batch Chemical Data Record is attached as Appendix A.

The selected doses of 400, 200, 100, 50, and 25 mg/kg were prepared by mixing a stock solution of the highest dose level and sequentially diluting from this stock to supply the remaining dose levels. The

diluent or vehicle was corn oil, Mazola brand. The stock solution was prepared by placing a weighed portion of naphthalene into a mixing graduated cylinder and adding corn oil to make up the proper volume. The mixture was mechanically stirred for a period of five minutes. Dilutions for rats were prepared so that the chemical would be delivered to the animal by gavage on the basis of one milliliter of the mixture per 200 grams body weight. Mixture quantities were prepared weekly. The actual quantities mixed were changed once (doubled) during the study to accommodate weight increases in the rats. The final mixtures were composed of the following:

Dose Level, mg/kg	Naphthalene, g	Serial Solution, ml	Corn Oil, ml	Total Volume, ml
400	21.6	--	Q.S.	270
200		135	135	270
100		135	135	270
50		135	135	270
25		135	135	270

For each dose level, the final mixtures were stored in glass vials at room temperature. Mixtures were prepared no more than seven days prior to use; gavage treatment was five consecutive dose days per week. Stainless steel gavage needles and tuberculin syringes graduated in 0.01 ml units were used to administer the gavage doses. One analysis for dose concentration accuracy was performed; results were within $\pm$ 10 percent of the concentrations for all dose levels except the 200 mg/kg which was 1.76 percent outside this range.

Animals

The study was performed with Fischer 344 rats received from Charles River, Portage, Michigan, on April 27, 1979, and held in quarantine 15 days. Dose administration began on May 14, 1979, and

continued five days a week for 13 weeks. The study concluded August 10, 1979. Copies of the Weekly Animal Report and BCL Miscellaneous Data Sheet are attached as Appendix B. Animals were examined daily during quarantine; no observable disease was detected prior to randomization. Five males and five females were taken at the time of randomization and necropsied; no internal lesions or disease were evident from this gross examination. The rats were randomized into test groups from the quarantine pool by tables of random numbers provided by Dr. James Joiner, Tracor Jitco, Incorporated. Sixty rats of each sex were distributed five per cage into 12 cages. The 12 cages were then distributed from a separate random numbers table to five dose levels and one control group per sex. The animals were individually marked by toe clipping. Male rats ranged in starting body weights from 170 to 215 grams, while females ranged from 135 to 155 grams.

Animal Maintenance

Rats were caged in groups of five animals in polycarbonate cages suspended from stainless steel racks, both manufactured by Lab Products, Incorporated. Automatic watering devices manufactured by Edstrum Industries provided water *ad lib*. Water was untreated at the laboratory and was supplied by the City of Columbus. Rats were housed in Room 7C-207 concurrently with mice also on subchronic Naphthalene study. Cages bedded with Absorb-Dri (Absorb-Dri, Incorporated) were changed twice weekly; rack shelves were covered with a spun-bonded polyester filter (DuPont 2024). Racks were changed once every two weeks. Animal feed was checked daily in cages and added as needed. Purina Lab Chow was used; a feed tag is appended to this report. Fresh feed and feeders were provided weekly. Animal rooms were maintained at 21-23 C and 40-60 percent relative humidity, 12 hours of fluorescent light, and 15 air changes per hour. There were 12 rat cages per 100 square feet of floor space; neither cage positions within the rack nor rack positions were rotated during the study.

Cages and feeders were cleaned in a continuous conveyor washer manufactured by Blakeslee, Chicago, Illinois. Cages received a one-wash and two-rinse sequence. "Exceed" brand detergent, manufactured by Economics Labs, St. Paul, Minnesota, was used in the wash sequence. Temperature of the rinse water was 180 F or greater. Racks were cleaned in a washer manufactured by Better Built, Saddlebrook, New Jersey. The rack washer had a single wash, double rinse sequence. The detergent and rinse temperature were the same as that used in the tunnel washer. The floor in Room 7C-207 was washed down daily with water and squeegeed dry.

Design of the Study

Five dose levels of 400, 200, 100, 50 and 25 mg/kg were administered to male and female rats by gavage at a rate of 0.5 milliliters per 100 grams of body weight. Vehicle controls received corn oil. Animals were gavaged in a hood five days a week for 13 weeks. Each dose level and control group contained ten rats of each sex.

The subchronic study began May 12, 1979; animals were observed twice each day for clinical signs of toxicity with at least six hours between observations. All clinical signs or negative observations were recorded daily in a bound observation book. Weekly individual animal weights and cage group food consumption were recorded. Day 91 of the study was August 10, 1979; surviving animals were terminated with CO_2 on August 14 and 15 with full necropsy and tissue collection.

Results

Survival

During the 13 weeks of the subchronic study all rats survived to scheduled termination except two 400 mg/kg males (individual animal numbers 4 and 9). These male rats died during the last week of the study. A survival table is attached as Appendix C.

Daily Observations

Observations were completed twice daily on all study animals, and clinical signs or negative observations were recorded daily. During the course of the 91 day study, clinical signs included diarrhea, lethargy, hunched posture, and rough coats. The highest dosed rats (400 mg/kg) had intermittent diarrhea, males showing this sign during weeks 3, 4, 10, and 13, and females during weeks 4, 7, 10, and 13. During the final week of the study the 400 mg/kg dosed animals were rough-coated; lethargic, and females had a hunched posture. Male rat number 9 showed all these clinical signs as well as becoming prone the day prior to death. During week 10 all rats among the three highest dose groups showed rough hair coats. No other signs of toxicity or clinical signs were noted during the 13 weeks of this subchronic study.

Diet Consumption and Body Weights

Cage group diet consumption was measured and recorded weekly. Total weekly group averages as well as individual daily consumption per group is included in the diet consumption table in Appendix D. No dose related trends are evident among any of the dosed groups or controls in either male or female rats.

Tables of body weight data are included in Appendix E. Individual animal weights were taken weekly during the subchronic study. An individual body weight summary table as well as an average weekly individual body weight table with percent differential weight changes compared to controls calculated are included. Graphs are also included, plotting average group body weight against time. The percent differential weight changes show a definite dose related trend among both male and female rats. The two highest dose groups, 400 and 200 mg/kg, of male rats have a greater than -10 percent differential weight; while the three highest dose groups (including 100 mg/kg) of female rats have a greater than -10 percent differential weight change. The most marked differences

in weight changes are in the highest male and female (400mg/kg) dosed groups. Weight changes were watched closely throughout this study, and, in order to guarantee against any dehydration effects, supplemental water dishes were provided daily to those rat groups gaining weight less rapidly than other groups.

Special Study - Hematology

The following analyses were performed on venous blood collected retroorbitally the day of scheduled termination; Hemoglobin (HgB), hematocrit (HCT), total and differential white blood cell count (WBC), red blood cell count (RBC), and mean corpuscular volume (MCV). The results are summarized in Appendix F. The mean value ($\bar{X}$), standard deviation (SD) and sample size (N) are presented for each parameter and each dosage group.

Among 400 mg/kg dosed male rats, a marginal decrease in the hemoglobin and hematocrit is apparent. Also apparent in this group of males are a moderate increase in absolute numbers of mature neutrophils (segs) and absolute decrease in lymphocytes with a reverse in the neutrophil/lymphocyte ratio, which results in the total WBC count being normal. Among female rats in this dose group, there is also a slight increase in the segmented neutrophils but the lymphocyte value appears normal. Females also have a marginal decrease in the hemoglobin and hematocrit values. Although statistical analyses were not performed on this data, the most pronounced effect appears to be in the 400 mg/kg male rats lymphocyte and neutrophil values.

PATHOLOGY

Rats

Methods:

Detailed necropsy examinations were performed on all rats in this study. The following tissues from all animals were placed in 10 percent neutral buffered formalin for fixation:

Mandibular lymph node	Heart
Salivary gland	Esophagus
Femur	Stomach
Thyroid	Uterus
Parathyroid	Brain (3 sections)
Small intestine	Thymus
Colon	Trachea
Liver	Pancreas
Prostate	Spleen
Testes	Kidneys
Ovaries	Adrenals
Lungs and mainstem bronchi	Urinary bladder
Mammary gland	Pituitary
Eyes	

Tissues listed above from animals in the control and highest dosage groups were processed in the routine manner, sectioned at 5 microns, stained with hematoxylin and eosin, and examined microscopically. In addition, kidneys from males and thymus glands from females in dosage group D (100 mg/kg) were examined microscopically since lesions which may have been compound induced were observed in these organs in animals of the respective sexes at the highest dosage group (400 mg/kg).

Results:

Necropsy observations for individual animals are shown in Tables 12 through 17 while a summary of necropsy observations is shown in Table 18. Lesions observed microscopically are shown in Tables 19 through 21. A summary of microscopic lesions by sex and dosage group is presented in Table 22.

Necrosis of renal tubular epithelium was observed in one male rat from the highest dosage group (400 mg/kg) which died spontaneously late in the study. There were no indications of degenerative changes in kidneys from other animals; however, the possibility that this lesion may have been induced by the test compound cannot be ruled out.

Depletion of thymic lymphocytes was observed in females from the highest dosage group (400 mg/kg). This may have been due to administration of the test compound since such changes are not common in control rats at this age. Neither of the changes described above occurred in rats of the respective sexes at the next lower dosage level (200 mg/kg).

All necropsy changes were considered to have been of spontaneous origin with no evidence that any resulted from compound administration. Necropsy observations generally correlated well with microscopic changes in animals which were examined microscopically.

Conclusions:

The only lesions which may have been induced by the test compound were renal tubular necrosis in one male rat at the highest dosage level (400 mg/kg) and depletion of thymic lymphocytes in two females from the highest dosage group (400 mg/kg). These lesions were not present in rats at the next lower dosage group (200 mg/kg).

Daryl C. Thake

Daryl C. Thake, D.V.M.
Veterinary Pathologist

TABLE 12. NECROPSY DATA BY INDIVIDUAL ANIMAL, NAPHTHALENE RATS, 0 mg/kg DOSE

Organ and Observation[a]	Sex	M	M	M	M	M	M	M	M	M	M	F	F	F	F	F	F	F	F	F	F
	Animal Number	S01	S02	S03	S04	S05	S06	S07	S08	S09	S10	S01	S02	S03	S04	S05	S06	S07	S08	S09	S10
	Pathology Number	796543	796544	796545	796546	796547	796548	796549	796550	796551	796552	796553	796554	796555	796556	796557	796558	796559	796560	796561	796562
Lung, all lobes hemorrhagic															P						
Ovarian sac, left, red, small amount of fluid														P							P

(a) P = present.

6394

000009

TABLE 13. NECROPSY DATA BY INDIVIDUAL ANIMAL, NAPHTHALENE RATS, 25 mg/kg DOSE

Organ and Observation[a]	Sex	M	M	M	M	M	M	M	M	M	M	M	F	F	F	F	F	F	F	F	F	F	F
	Animal Number	S01	S02	S03	S04	S05	S06	S07	S08	S09	S10	S01	S02	S03	S04	S05	S06	S07	S08	S09	S10
	Pathology Number	796643	796644	796645	796646	796647	796648	796649	796650	796651	796652	796653	796654	796655	796656	796657	796658	796659	796660	796661	796662
Liver, middle lobe, small nodule at diaphragmatic hiatal area															P						
Ovary, left, small amount of fluid in paraovarian sac																P					
Ovary, right, small paraovarian cyst																	P				
Uterine horns, filled with clear fluid (estrus)												P			P			P			

(a) P = present.

000010

6394

TABLE 15. NECROPSY DATA BY INDIVIDUAL ANIMAL, NAPHTHALENE RATS, 100 mg/kg DOSE

Organ and Observation[a]	Sex	M	M	M	M	M	M	M	M	M	M	F	F	F	F	F	F	F	F	F	F	F
	Animal Number	S01	S02	S03	S04	S05	S06	S07	S08	S09	S10	S01	S02	S03	S04	S05	S06	S07	S08	S09	S10
	Pathology Number	796603	796604	796605	796606	796607	796608	796609	796610	796611	796612	796613	796614	796615	796616	796617	796618	796619	796620	796621	796622
Eye, left, yellow material in anterior chamber or on lens													P								
Liver, middle lobe, small nodule at diaphragmatic hiatal area																				P	
Periovarian fat, 5mm red nodule on fibrous stalk																	P				
Uterine horns, filled with clear fluid (estrus)												P			P				P		

(a) P = present.

6394

000011

TABLE 16. NECROPSY DATA BY INDIVIDUAL ANIMAL, NAPHTHALENE RATS, 200 mg/kg DOSE

Organ and Observation[a]	Sex	M	M	M	M	M	M	M	M	M	M	F	F	F	F	F	F	F	F	F	F
	Animal Number	S01	S02	S03	S04	S05	S06	S07	S08	S09	S10	S01	S02	S03	S04	S05	S06	S07	S08	S09	S10
	Pathology Number	796583	796584	796585	796586	796587	796588	796589	796590	796591	796592	796593	796594	796595	796596	796597	796598	796599	796600	796601	796602
Liver, middle lobe, nodular protrusion into hiatus of diaphragm								P													
Uterine horns, filled with clear fluid (estrus)															P		P				
Uterine horns, slightly reddened and slightly enlarged (estrus)																		P			

(a) P = present.

000012

6394

TABLE 19. HISTOPATHOLOGY DATA BY INDIVIDUAL ANIMAL, NAPHTHALENE RATS, 0 mg/kg DOSE

Organ and Diagnosis(a)	Sex	M	M	M	M	M	M	M	M	M	M	F	F	F	F	F	F	F	F	F	F
	Animal Number	S01	S02	S03	S04	S05	S06	S07	S08	S09	S10	S01	S02	S03	S04	S05	S06	S07	S08	S09	S10
	Pathology Number	796543	796544	796545	796546	796547	796548	796549	796550	796551	796552	796553	796554	796555	796556	796557	796558	796559	796560	796561	796562
Bone marrow, reticulum cell hyperplasia, focal												2							2		
Colon, parasitosis					P										P			P			
Eye, retina, retinal rosette		P																			
Eye, retina, focus of vacuolated cells		P																			
Heart, papillary muscle, histiocytic infiltrate, focal						2															
Heart, myocarditis, subacute, multifocal									2												
Heart, left ventricle, myocardium, myocarditis, fibrosis, focal										2											
Heart, myocardium, myocarditis, multifocal																			2		
Liver, centrilobular areas, vacuolization, diffuse							2		2		2										
Liver, centrilobular areas, hepatocellular vacuolization, diffuse								2													
Pleural cavity, mast cell and neutrophil accumulation													2								

6394

000013

TABLE 19. (Continued)

Organ and Diagnosis(a)	Sex	M	M	M	M	M	M	M	M	M	M	F	F	F	F	F	F	F	F	F	F
	Animal Number	S01	S02	S03	S04	S05	S06	S07	S08	S09	S10	S01	S02	S03	S04	S05	S06	S07	S08	S09	S10
	Pathology Number	796543	796544	796545	796546	796547	796548	796549	796550	796551	796552	796553	796554	796555	796556	796557	796558	796559	796560	796561	796562
Pleural cavity, inflammation, fibrinous															2						
Thyroid gland, lymphocytic infiltrate, focal							1														
Trachea, tracheitis, acute																					2

(a) Severity rating: 1 = minimal, 2 = mild, 3 = moderate, 4 = severe, P = present.

6394

000014

TABLE 20. HISTOPATHOLOGY DATA BY INDIVIDUAL ANIMAL, NAPHTHALENE RATS, 200 mg/kg DOSE, MALE KIDNEY TISSUES ONLY AND FEMALE THYMUS TISSUES ONLY

Organ and Diagnosis(a)	Sex	M	M	M	M	M	M	M	M	M	M	F	F	F	F	F	F	F	F	F	F
	Animal Number	S01	S02	S03	S04	S05	S06	S07	S08	S09	S10	S01	S02	S03	S04	S05	S06	S07	S08	S09	S10
	Pathology Number	796583	796584	796585	796586	796587	796588	796589	796590	796591	796592	796593	796594	796595	796596	796597	796598	796599	796600	796601	796602
Kidney, cortex, lymphocytic infiltrate, focal											1										
Kidney, tubular regeneration, focal			1																		

(a) Severity rating: 1 = minimal, 2 = mild, 3 = moderate, 4 = severe, P = present.

6394

000015

TABLE 21. HISTOPATHOLOGY DATA BY INDIVIDUAL ANIMAL, NAPHTHALENE RATS, 400 mg/kg DOSE

Organ and Diagnosis(a)	Sex	M	M	M	M	M	M	M	M	M	M	F	F	F	F	F	F	F	F	F	F	F
	Animal Number	S01	S02	S03	S04	S05	S06	S07	S08	S09	S10	S01	S02	S03	S04	S05	S06	S07	S08	S09	S10
	Pathology Number	796563	796564	796565	796566	796567	796568	796569	796570	796571	796572	796573	796574	796575	796576	796577	796578	796579	796580	796581	796582
Adrenal glands, congestion										3											
Kidney, cortex, tubular degeneration, diffuse										3											
Liver, centrilobular areas, hepatocellular vacuolization, multifocal						1															
Liver, subcapsular area, fibrosis, focal							2														
Lung, pneumonia, granulomatous, focal, gavage induced												2									
Lung, pneumonia, histiocytic, focal or multifocal, gavage induced																			2	1	
Lung, pneumonia, subacute, focal																1					
Parathymic adipose tissue, congestion and hemorrhage					3																
Thymus, lymphoid depletion																3					3

(a) Severity rating: 1 = minimal, 2 = mild, 3 = moderate, 4 = severe, P = present.

6394-1

010016

TABLE 18. SUMMARY OF NECROPSY DATA BY GROUP AND SEX, NAPHTHALENE RATS

Organ and Observation	Dose Group	0 mg/kg		25 mg/kg		50 mg/kg		100 mg/kg		200 mg/kg		400 mg/kg	
	Sex	M	F	M	F	M	F	M	F	M	F	M	F
	Number in Group	10	10	10	10	10	10	10	10	10	10	10	10
Anus, inflamed												1	
Eye, left, yellow material in anterior chamber or on lens									1				
Heart, enlarged												1	
Liver, middle lobe, small nodule at diaphragmatic hiatal area					1		1		1				
Liver, middle lobe, nodular protrusion into hiatus of diaphragm										1			
Lung, all lobes hemorrhagic			1										
Ovarian sac, left, red, small amount of fluid			2										
Ovary, left, small amount of fluid in parovarian sac					1								
Ovary, right, small parovarian cyst					1								
Periovarian fat, 5 mm red nodule on fibrous stalk									1				
Seminal vesicles, small												3	
Testes, two-thirds normal size						1							
Uterine horns filled with clear fluid (estrus)					3		3		3		2		
Uterine horns, slightly reddened and slightly enlarged (estrus)											1		

6394

000027

TABLE 22. SUMMARY OF HISTOPATHOLOGY DATA BY GROUP AND SEX, NAPHTHALENE RATS

Organ and Diagnosis	Dose Group	0 mg/kg		200 mg/kg		400 mg/kg	
	Sex	M	F	M	F	M	F
	Number in Group	10	10	10	10	10	10
Adrenal glands, congestion						1	
Bone marrow, reticulum cell hyperplasia, focal			2				
Colon, parasitosis		1	2				
Eye, retina, retinal rosette		1					
Eye, retina, focus of vacuolated cells		1					
Heart, papillary muscle, histiocytic infiltrate, focal		1					
Heart, left ventricle, myocardium, myocarditis, fibrosis, focal		1					
Heart, myocarditis, subacute, multifocal		1					
Heart, myocardium, myocarditis, multifocal			1				
Kidney, cortex, lymphocytic infiltrate, focal				1			
Kidney, cortex, tubular degeneration, diffuse						1	
Kidney, tubular regeneration, focal				1			
Liver, centrilobular areas, vacuolization, diffuse		3					
Liver, centrilobular areas, hepatocellular vacuolization, multifocal or diffuse		1				1	
Liver, subcapsular area, fibrosis, focal						1	
Lung, pneumonia, granulomatous, focal, gavage induced							1

6394

000018

TABLE 22. (Continued)

Organ and Diagnosis	Dose Group	0 mg/kg		200 mg/kg		400 mg/kg	
	Sex	M	F	M	F	M	F
	Number in Group	10	10	10	10	10	10
Lung, pneumonia, histiocytic, focal or multifocal, gavage induced							2
Lung, pneumonia, subacute, focal							1
Parathymic adipose tissue, congestion and hemorrhage						1	
Pleural cavity, mast cell and neutrophil accumulation			1				
Pleural cavity, inflammation, fibrinous			1				
Thymus, lymphoid depletion							2
Thyroid gland, lymphocytic infiltrate, focal		1					
Trachea, tracheitis, acute			1				

6394

000019

APPENDIX A

BATCH CHEMICAL DATA RECORD
MRI ANALYTICAL REPORT

6394

DATA RECORD

Columns 1 through 15 apply to each card on this page.

								8-13						14 -
7	2	3	2	7	0	C	5	2	9	0	4	0		

ITEM			CARD COL.
SECTION I			
1. Chemical Common Name Naphthalene			
2. Name of Manufacturer Fisher Scientific Company	FOR OFFICE USE (Do Not Code)		25 - 27
3. Chemical Name Used in Catalog Naphthalene Scintanalyzed			
4. Manufacturer's Lot Number of Batch 775379			
5. Is batch labeled? (e.g. isotope)	1 - Yes* 2 - No	2	28
6. Is there a reserve supply of batch?	1 - Yes 2 - No	1	29
7. Is batch purity tested?	1 - Yes *(Results of analysis)** 2 - No	1	30
8. Physical State of Chemical Batch		1	31
1 - Solid *(Indicate melting range in Item 9)* 2 - Liquid *(Indicate boiling range in Item 9)* 3 - Gel *(Indicate liquification range in Item 9)* 4 - Gas *(Skip Item No. 9)*			

ITEM		CARD C
9. Critical Temperature Range *(Degrees Centigrade)*	8 0 0 TO 8 1 0	32 - 3
10. How was the physical property obtained?	1 - Observed 2 - Literature 3 - Manufacturer's Specifications	1	40
SECTION II		
The following information should be provided only if available. Enter "1" in appropriate boxes and report data in Comment area.		
11. Date Received from Supplier*		1	41
12. Date of Manufacture and Original Storage by Manufacturer*			42
13. Methods of Preparation or Synthesis*			43
14. Special Treatment Applied After Receipt *(e.g. purification methods)**			44
CARD NUMBER		B	80

COMMENTS: Refer to Item No. when making comments.

Bioassayer: Battelle Research Institute

11. 3/28/78

I. BULK CHEMICAL STUDIES

B. ANALYSIS: The elemental analysis for carbon was slightly low while that for hydrogen was slightly high. Thin layer chromatography using two developing systems indicated only the major component. Vapor phase chromatography by one system indicated one impurity with a relative area of 0.10% of the major peak area. A second vapor phase chromatographic system indicated two impurities (0.13 and 0.02% of the major peak area). Naphthalene was found to contain 0.23 $\pm$ 0.04(δ)% water by titration with Karl Fischer reagent. The ultraviolet, infrared and nuclear magnetic resonance spectra were consistent with literature spectra.

C. HEAT STABILITY: Naphthalene is stable when stored as the bulk chemical for a period of 2 weeks at temperatures of up to 60°C.

II. CHEMICAL/VEHICLE STUDIES

A. ACUTE TOXICITY (LD_{50}) DOSAGE MIXING PROTOCOL: Naphthalene can be suspended in 0.5% aqueous methyl cellulose, and the suspension remains visually homogeneous for a maximum of 1 min, if the dry chemical is first ground in a mortar and the naphthalene/methyl cellulose mixture is sonicated for 30 min. When the mixture separates, it can be rehomogenized by treatment with the vortex mixer for 1 min.

* COMMENT REQUIRED

II. CHEMICAL/VEHICLE STUDIES (Concluded)

B. CHRONIC LEVEL STABILITY STUDY: Naphthalene is stable when dissolved in corn oil at a dose level of 4% and stored at room temperature for 7 days.

III. ANALYSIS PROTOCOL FOR USE BY BIOASSAYERS

A. BULK CHEMICAL

1. GENERAL PROTOCOL: The objective is to determine whether a given compound as received and stored is, and remains, identical to that received and analyzed by MRI.

The most efficient method for determining stability is to maintain a stable standard to be used for comparison with the stored bulk chemical. Therefore, when you receive the bulk shipment, remove standard samples of 1 g for solids or 2 ml for liquids for each testing time.

Place each sample in a glass vial with a Teflon-lined top tightly closed and, if possible, sealed. Place samples in a freezer at -20°C for storage prior to analysis.

Remove a single standard vial from the freezer approximately 4 hr prior to analysis. Perform analyses in tandem so that test sample results can be compared to the standard. Also compare the results obtained in the MRI analytical reports. Slight differences may occur between the MRI report and your analyses. However, your standard and sample analyses should be identical to each other and identical to MRI data within expected variation limits.

2. PURITY: Vapor-phase chromatography (this report, Section I-B-1-d-System 2; 10% Carbowax 20 MTPA).

3. IDENTITY: Infrared spectroscopy (this report, Section I-B-2-b-(1)).

B. CHEMICAL/VEHICLE: Follow the analysis and extraction procedures described above in Sections II-B-2 and II-B-3 for the chronic level 7-day stability study.

IV. STORAGE CONDITIONS

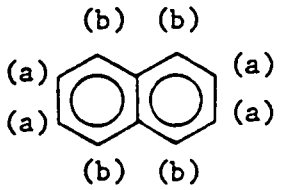
Naphthalene should be stored in sealed containers at room temperature away from oxidizing materials and sources of ignition. The bulk material is stable for 2 weeks at temperatures of up to 60°C, indicating a stability of at least 24 weeks at room temperature (25°C). Naphthalene was also found to be stable for 7 days at 25°C when dissolved in corn oil at a dose level of 4%. Naphthalene dust forms an explosive mixture in air at high concentrations; therefore, care should be exercised when preparing to open storage vessels containing naphthalene.

V. HANDLING PROTOCOL

Naphthalene is irritating to the eyes and skin, and is toxic if ingested. Acute overexposure may cause dermatitis, headache, and nausea. Chronic overexposure causes renal shutdown and eye cataracts. This material is also suspected of causing neoplasms in rats. Thus, all personnel should be fully protected when working with naphthalene. Coveralls, gloves, eye and foot protection are necessary. Respiratory protection should be provided in areas where naphthalene dust may form. Dust type cannister or air-supplied masks are suitable. AN work should be conducted in well-ventilated areas. Although we did not encounter this problem, it is very possible to create a dust when handling solid naphthalene. Care should be taken to prevent this since high concentrations of naphthalene dust are explosive in air.

MIDWEST RESEARCH INSTITUTE
TRACOR JITCO, INC. SUBCONTRACT NO. 74-24-106002
September 19, 1978

COMPOUND: Naphthalene



$C_{10}H_8$

SUBMITTED BY: Tracor Jitco
CBDS NO.: C52904
MRI REQUEST NO.: 335
PROJECTED USE: Bioassay studies at Battelle Research Institute
TOXICITY:1/ orl-chd LDL_o:100 mg/kg
orl-hmn LDL_o: 50 mg/kg
orl-rat LD_{50}:1,780 mg/kg
scu-rat TDL_o:3,500 mg/kg/98DI TFX:NEO
AQTX: Aquatic Toxicity Rating: TLm96:10-1 ppm

TRACOR JITCO P.O. NO.: 3274
P.O. DATE: 3-17-78
RECEIPT DATE: 3-28-78
MANUFACTURER DATA
COMPANY: Fisher Scientific Company
SIZE: 48 x 1 lb
LOT NO.: 775379
BATCH NO.: 01
PURITY: Scintillation grade
ANALYTICAL DATA: None enclosed

I. BULK CHEMICAL STUDIES

A. HOMOGENIZATION

The contents of the 48 bottles were combined in a fiberboard drum and the drum shaken and rolled for 5 min. Samples were taken and the remainder of the naphthalene returned to the original containers.

1/ Fairchild, Edward J., Ph.D. (ed.), "Registry of Toxic Effects of Chemical Substances," U.S. Department of Health, Education and Welfare, Cincinnati, Ohio, p. 566 (1977).

B. ANALYSIS

1. ASSAY

a. CHROMATOGRAPHY-TLC

Plates: Silica gel F-254, 0.25 mm layer, precoated

Ref. Standard: Pyrene (1 µl of a 10 µg/µl solution in acetone)

Amount spotted: 100 and 300 µg (10 and 30 µl of a 10 µg/µl solution in acetone)

Visualization: UV (254 nm) and iodine vapor

System 1: Hexanes (100%)

R_f: 0.62
R_{st}: 1.29

System 2: Pentane:carbon tetrachloride (50:50)

R_f: 0.75
R_{st}: 1.08

b. ELEMENTAL ANALYSIS

Element	C	H
Theory	93.70	6.30
Determined	93.06	6.59
	92.87	6.70
% D/T	99.22	105.48

c. WATER ANALYSIS
(Karl Fischer)

0.23 ± 0.04(δ)%

d. VAPOR PHASE CHROMATOGRAPHY

Instrument: Varian 3740
Detector: Flame ionization
Inlet temperature: 200°C
Detector temperature: 250°C
Carrier gas: Nitrogen
Carrier flow rate: 70 cc/min

(1) System 1

Column: 3% SP2100 on 100/120 Supelcoport, 1.8 m x 4 mm ID; glass
Oven temperature program: 5 min at 50°C, then 50 to 250°C at 10°C/min
Sample injected: 5 µl of a 10 mg/ml solution in methylene chloride to quantitate impurities and 5 µl of a 5 mg/ml solution in methylene chloride to check for detector overloading

Results: Major peak and one impurity after the major peak which had an area of 0.1%, relative to the area of the major peak.

Peak	Retention Time (min)	Retention Time (Relative to Naphthalene)	Area % (Relative to Naphthalene)
1	12.0	1.00	100
2	13.9	1.16	0.10

(2) System 2

Column: 10% Carbowax 20M-TPA on 80/100 Chromasorb W (AW); 1.8 m x 4 mm ID; glass
Oven temperature program: 5 min at 50°C, then 50 to 200°C at 10°C/min
Sample injected: 5 µl of a 10 mg/ml solution in methylene chloride to quantitate impurities and 5 µl of a 5 mg/ml solution in methylene chloride to check for detector overloading.

Results: Major peak and two impurities, one before and one after the major peak. The impurities had a combined area of 0.15%, relative to the area of the major peak.

Peak	Retention Time (min)	Retention Time (Relative to Naphthalene)	Area % (Relative to Naphthalene)
1	12.3	0.96	0.02
2	12.8	1.00	100
3	14.2	1.10	0.13

2. IDENTITY (major component only unless (a) mixture specified or (b) major component not nominal ingredient)

a. PHYSICAL PROPERTIES

Determined	Literature Values
(1) Appearance: White crystalline solid	
(2) m.p.: 80-81°C (visual, capillary)	80.2°C[2/]

b. SPECTRAL DATA

(1) Infrared

Instrument: Beckman IR-12 Cell: salt melt Results: See attached spectrum	Consistent with literature spectrum[3/]

(2) Ultraviolet/Visible

Instrument: Cary 118 No absorbance between 350 and 800 nm (visible region) at a concentration of 9.3 mg/ml	4/

λ_{max} (nm)	$\varepsilon \times 10^{-2}$	λ_{max} (nm)	$\varepsilon \times 10^{-2}$
312	1.779 ± 0.018(δ)	310	2.922
286	36.32 ± 0.16(δ)	285	42.20
275	54.51 ± 0.21(δ)	273	65.64
265	48.86 ± 0.16(δ)	265	57.83
258 (shoulder)	34.02 ± 0.12(δ)	257	38.55
221	1,022.63 ± 6.82(δ)	221	1,417 16
Solvent: Hexane		Solvent: Cyclohexane	

2/ Windholz, Martha, et al., eds., The Merck Index, Merck and Company, Inc., Rahway, New Jersey, Ninth Edition, p. 828 (1976).

3/ "Sadtler Standard Spectra," Sadtler Research Laboratories, Philadelphia, Pennsylvania, IR No. 865.

4/ "Sadtler Standard Spectra," Sadtler Research Laboratories, Philadelphia, Pennsylvania, UV No. 265.

Instrument: Varian 3740 (autosampler)
Detector: Flame ionization
Inlet temperature: 200°C
Detector temperature: 300°C
Carrier gas: Nitrogen
Carrier flow rate: 70 cc/min
Column: 3% SP2100 on 100/120 Supelcoport; 1.8 m x 4 mm ID; glass
Oven temperature program: 80°C, isothermal
Retention times: Naphthalene 6.3 min
Decane (internal standard) 9.7 min

3. RESULTS

Storage Temperature	Recovery (% of -20°C Sample)
-20°C	100.0 ± 0.4(δ)
5°C	100.0 ± 0.4(δ)
25°C	100.4 ± 0.4(δ)
60°C	100.2 ± 0.4(δ)

4. CONCLUSIONS

Naphthalene is stable when stored as the bulk chemical for a period of 2 weeks at temperatures of up to 60°C.

II. CHEMICAL/VEHICLE STUDIES

A. ACUTE TOXICITY (LD_{50}) DOSAGE MIXING PROTOCOL

1. TYPICAL DOSAGE LEVEL

The dosage concentration was calculated to be 240 mg/ml using the following data:

Weight of test animal: 100 g
LD_{50}: 2,400 mg/kg, orl-rat[6/]
Volume to be administered: 1 ml
Solvent: 0.5% methyl cellulose in water

6/ Safety and Toxicity Data for naphthalene prepared by Tracor Jitco, Inc., dated March 3, 1978.

2. MIXING

Naphthalene would not dissolve or suspend in water or corn oil at the above concentration. However, the compound would suspend in 0.5% aqueous methyl cellulose.

Naphthalene was ground in a mortar before weighing 720 mg into a 5 ml vial. Aqueous methyl cellulose (0.5%) was added to bring the total volume to 3 ml. The mixture was sonicated 15 min, placed on a vortex mixer for 2 min, and sonicated again for 15 min. This procedure produced a suspension which remained visually uniform for at least 1 min and could be drawn through an 18-gauge gavage needle.

When the uniform mixture began to separate, it could be rehomogenized by treatment with the vortex mixer for 1 min.

3. CONCLUSIONS

Naphthalene can be suspended in 0.5% aqueous methyl cellulose, and the suspension remains visually homogeneous for a maximum of 1 min, if the dry chemical is first ground in a mortar and the naphthalene/methyl cellulose mixture is sonicated for 30 min. When the mixture separates, it can be rehomogenized by treatment with the vortex mixer for 1 min.

B. CHRONIC LEVEL STUDIES - SEVEN-DAY, ROOM TEMPERATURE STABILITY IN CORN OIL AT A 4% DOSE LEVEL

1. SAMPLE PREPARATION AND STORAGE

Naphthalene (2.139 ± 0.001 g) was placed in a 50-ml volumetric flask and diluted to the volume mark with corn oil (44.357 ± 0.001 g). The chemical dissolved upon sonicating for 5 min to give a solution of 46.00 ± 0.02 mg/g or 42.78 ± 0.02 mg/ml (4.29% w/v or 4.60% w/w).

As soon as the solution had been prepared, eight accurately weighed 1.60 g aliquots were removed and sealed in separate 60-ml septum vials (Microsep F-138 gas chromatography septa with Teflon® film facing, from Canton Biomedical Products, Inc.; aluminum crimp seals from Wheaton Scientific Company, Inc.). Duplicate aliquots were used as initial, or zero-time, samples and for storage at 1, 5 and 7 days, respectively.

2. SAMPLE EXTRACTION AND ANALYSIS

Extracting solvent containing an internal reference standard was prepared by weighing 4.571 ± 0.0001 g of 1,2-bis(2-methoxyethoxy)ethane,

transferring to a 1-liter volumetric flask, and diluting to the mark with absolute methanol. Concentration of reference standard: 4.571 ± 0.0002 mg/ml.

To extract each sample aliquot, the septum vial was opened, 50 ml of the extracting solvent was added by volumetric pipette, and the vial immediately resealed. The corn oil/methanol mixture was manually shaken for 15 sec, sonicated 30 sec, and 10 ml of the resulting suspension was decanted into a 12-ml centrifuge tube and centrifuged for 5 min. A portion of the clear, methanolic supernatant solution (5 ml) was then transferred to an 8.5 ml septum vial for subsequent analysis by the gas chromatographic system outlined below:

Instrument: Bendix 2500 with Hewlett-Packard 3380A Automatic Integrator
Column: 10% SP-2330 on 100/120 mesh Chromosorb W(AW), 1.8 m x 2 mm ID, glass, silanized
Detection: Flame ionization
Temperatures: Inlet, 180°C
Oven, 125°C, isothermal
Detector: 220°C
Carrier gas: Nitrogen
Flow rate: 30 cc/min
Volume of solution injected: 4 µl
Retention times: Test chemical, 6.0 min
Reference standard, 8.3

3. QUALITY CONTROL PROTOCOLS

Analyses were performed in duplicate using 1,2-bis(2-methoxyethoxy)-ethane as an internal reference standard. Recovery studies were performed in duplicate at the same concentration level as the test samples. Gas chromatographic linearity was determined with standard solutions in methanol at 1.84, 1.38 and 0.72 mg/ml concentrations for the naphthalene and 5.06, 3.79, and 2.53 mg/ml for the internal reference. The least squares plot correlation coefficients were > 0.999 for both the test chemical and the internal reference (effectively 1.0, linear).

4. RESULTS

Storage Time (days)	Average % (w/w) Chemical Found in Chemical/Vehicle Mixtures[a,b]
1	4.57 ± 0.07[c]
5	4.53 ± 0.07
7	4.58 ± 0.07

a/ Corrected for zero-time recovery yield of 94.4 ± 0.8%.
b/ Theoretical concentration of chemical in corn oil, 4.601 ± 0.002% (w/w).
c/ The error values in this table are average deviations obtained in the analytical measurements of the test solutions.

5. CONCLUSION

Naphthalene is stable when dissolved in corn oil at a dose level of 4% and stored at room temperature for 7 days.

III. ANALYSIS PROTOCOL FOR USE BY BIOASSAYERS

A. BULK CHEMICAL

1. GENERAL PROTOCOL

The objective is to determine whether a given compound as received and stored is, and remains, identical to that received and analyzed by MRI.

The most efficient method for determining stability is to maintain a stable standard to be used for comparison with the stored bulk chemical. Therefore, when you receive the bulk shipment, remove standard samples of 1 g for solids or 2 ml for liquids for each testing time.

Place each sample in a glass vial with a Teflon-lined top tightly closed and, if possible, sealed. Place samples in a freezer at -20°C for storage prior to analysis.

Remove a single standard vial from the freezer approximately 4 hr prior to analysis. Perform analyses in tandem so that test sample results can be compared to the standard. Also compare the results obtained in the MRI analytical reports. Slight differences may occur between the MRI report and your analyses. However, your standard and sample analyses should be identical to each other and identical to MRI data within expected variation limits.

2. PURITY

Vapor-phase chromatography (this report, Section I-B-1-d-System 2; 10% Carbowax 20 MTPA).

3. IDENTITY

Infrared spectroscopy (this report, Section I-B-2-b-(1)).

B. CHEMICAL/VEHICLE

Follow the analysis and extraction procedures described above in Sections II-B-2 and II-B-3 for the chronic level 7-day stability study.

IV. STORAGE CONDITIONS

Naphthalene should be stored in sealed containers at room temperature away from oxidizing materials and sources of ignition. The bulk material is stable for 2 weeks at temperatures of up to 60°C, indicating a stability of at least 24 weeks at room temperature (25°C). Naphthalene was also found to be stable for 7 days at 25°C when dissolved in corn oil at a dose level of 4%. Naphthalene dust forms an explosive mixture in air at high concentrations; therefore, care should be exercised when preparing to open storage vessels containing naphthalene.

V. HANDLING PROTOCOL

Naphthalene is irritating to the eyes and skin, and is toxic if ingested. Acute overexposure may cause dermatitis, headache, and nausea. Chronic overexposure causes renal shutdown and eye cataracts. This material is also suspected of causing neoplasms in rats. Thus, all personnel should be fully protected when working with naphthalene. Coveralls, gloves, eye and foot protection are necessary. Respiratory protection should be provided in areas where naphthalene dust may form. Dust type cannister or air-supplied masks are suitable. AN work should be conducted in well-ventilated areas. Although we did not encounter this problem, it is very possible to create a dust when handling solid naphthalene. Care should be taken to prevent this since high concentrations of naphthalene dust are explosive in air.

MIDWEST RESEARCH INSTITUTE

Bulk Chemical Studies

Mike Cannon
Junior Chemist

Linda Siemann
Junior Chemist

Dave Steele
Assistant Chemist

Richard Brown
Associate Chemist

Chemical Vehicle Studies

Lorren D. Kurtz
Junior Chemist

Bruce Schworer
Junior Chemist

John Rollheiser
Associate Chemist

Thornton C. Carpenter, Ph.D.
Associate Chemist

Approved:

Evelyn Murrill, Ph.D.
Senior Advisor for Chemistry

E. J. Woodhouse, Ph.D.
Principal Chemist

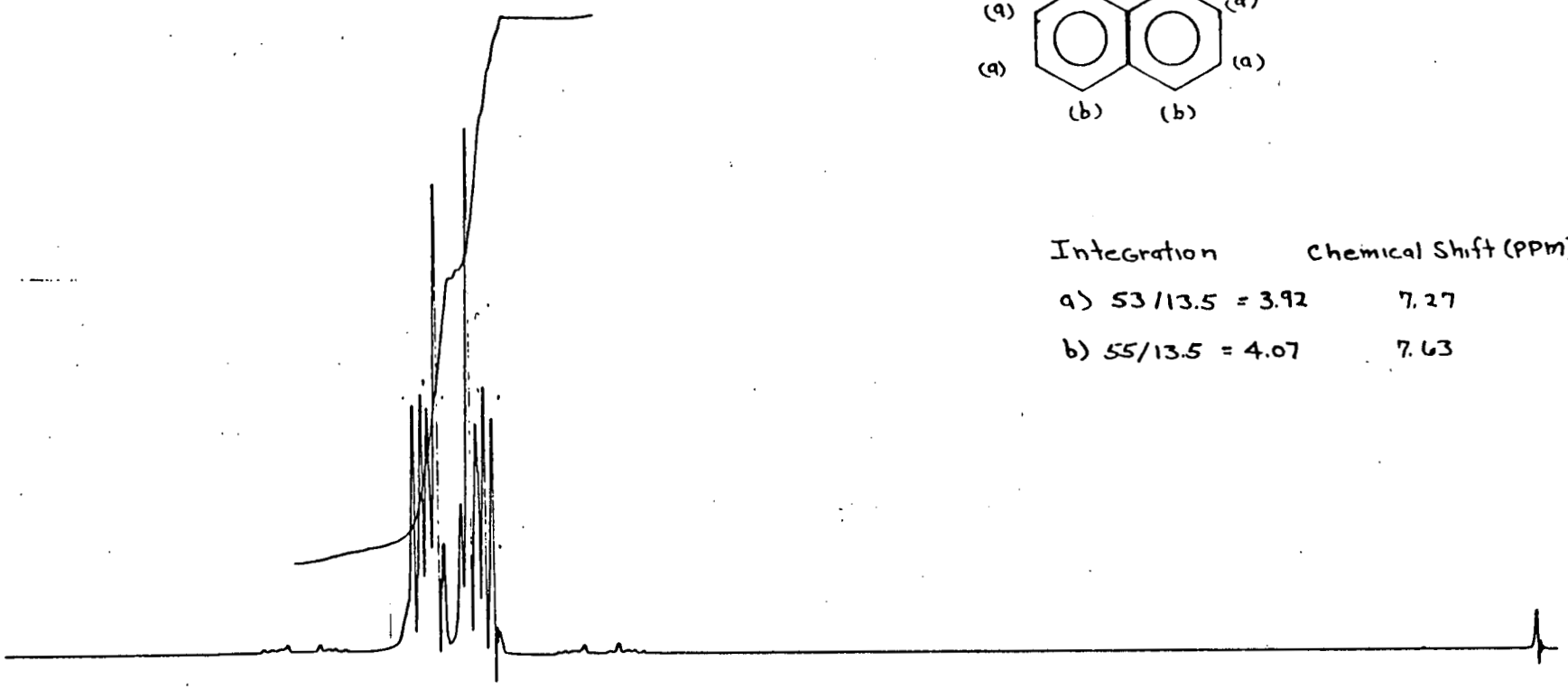
APPENDIX B

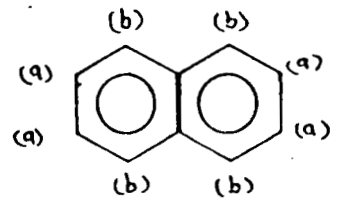
WEEKLY ANIMAL REPORT
BCL MISCELLANEOUS DATA SHEET

639

START OF SWEEP

END OF SWEEP





Integration	Chemical Shift (PPM)
a) 53/13.5 = 3.92	7.27
b) 55/13.5 = 4.07	7.63

Sweep offset 5 PPM

(8) 10 9 8 7 6 5 4 3 2 1 0

SPECTRUM AMPL. 10x8

FILTER 1 sec

RF POWER .05 mG

SWEEP TIME 5 min

SWEEP WIDTH 10 ppm or Hz

END OF SWEEP 0 ppm or Hz

SAMPLE: Naphthalene

335

LOT 775379
BATCH 01

SOLVENT: Chloroform-d

REMARKS:

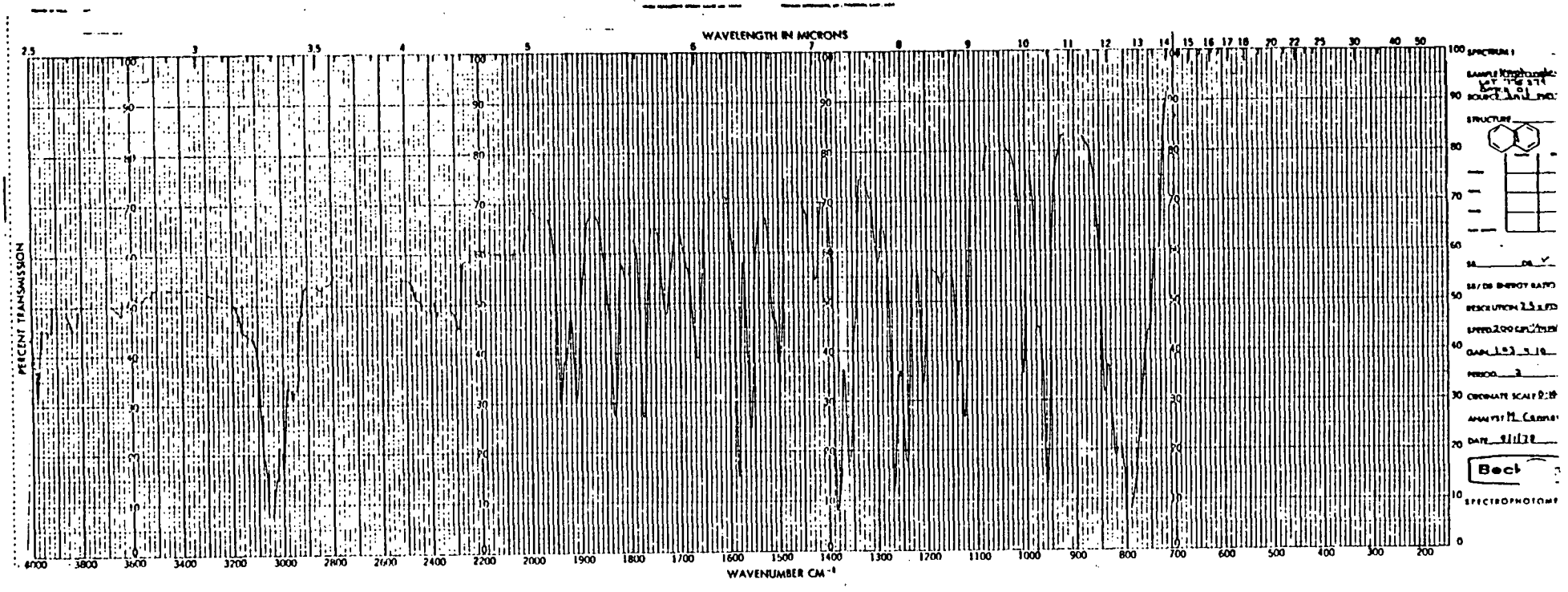
OPERATOR P. Seger

DATE 6/30/78

SPECTRUM NO. 335

EM-360 60 MHz NMR SPECTROMETER 000033

6394


WAVELENGTH IN MICRONS
PERCENT TRANSMISSION
WAVENUMBER CM⁻¹
SPECTRUM 1
STRUCTURE
SPECTROPHOTOMETER

000034

CARCINOGENESIS BIOASSAY DATA SYSTEM
WEEKLY ANIMAL REPORT

LABORATORY IDENTIFICATION			
1. NCI CONTRACT NUMBER: N01-CP-1-06002	2. LABORATORY: Battelle Columbus Laboratories		4. TODAY'S DATE: April 27, 1979
	3. PROJECT MANAGER: Dr. Arthur C. Peters	3a. PRINCIPAL INVESTIGATOR: Dr. Arthur C. Peters	

ANIMAL IDENTIFICATION	5. STRAIN OR STOCK	B6C3F1 Mice		Fischer 344 Rat									
	6. SPECIAL REQUIREMENTS	DOB-3/13/79		DOB-3/27/79									
	7. SUPPLIER*	CRPM		CRPM									
	8. SEX	♂	♀	♂	♀	♂	♀	♂	♀	♂	♀	♂	♀
ANIMALS RECEIVED THIS WEEK	9. DATE SHIPPED	4/27/79	4/27/79	4/27/79	4/27/79								
	10. DATE RECEIVED	4/27/79	4/27/79	4/27/79	4/27/79								
	11. NUMBER SHIPPED	75	75	75	75								
	12. NUMBER RECEIVED	75	75	75	75								
	13. BOXES SHIPPED	2	2	3	3								
	14. BOXES RECEIVED	2	2	3	3								
	NO. DISCARDED ON ARRIVAL — 15. DEAD	1	—	—	—								
	NO. DISCARDED ON ARRIVAL — 16. SICK	—	—	—	—								
	17. BALANCE (No. Received Minus No. Discarded)	74	76	75	75								
ANIMALS DISCARDED FROM HOLDING CAGES DURING QUARANTINE THIS WEEK	18. DEAD												
	19. SICK												
	20. UNDERWEIGHT												
	21. OVERWEIGHT												
	22. OVERAGE												
	23. OTHER												
	24. TOTAL DISCARDED												
ANIMALS USED THIS WEEK	25. TESTING												
	26. SURPLUS												
	27. TOTAL USED												

0394

000025

BCL MISCELLANEOUS DATA SHEET

NAME OF CHEMICAL: Naphthalene C 52904 BCL 28

CHEMICAL PURITY: 99.6 %

TYPE OF STUDY: Subchronic TREATMENT DATES: May 14-August 10,1979

OBSERVATION DATES: August 11, 1979 to August 14, 1979 ; 2-4 days

RANDOMIZED BODY WEIGHT RANGES USED IN TEST:

Mice ♀ 18-21 g Mice ♂ 22-28 g Age 8 weeks

Rats ♀ 135-155 g Rats ♂ 170-215 g Age 6 weeks

REMARKS:

Date of Birth:

Mice March 13, 1979

Rats March 27, 1979

APPENDIX C

SURVIVAL TABLE

SURVIVAL TABLE - RATS

Chemical: Naphthalene

Study: Subchronic

Dates: May 11 through August 10, 1979

Dose, mg/kg	Week of Study 0	1	2	3	4	5	6	7	8	9	10	11	12	13
Males														
400	10	10	10	10	10	10	10	10	10	10	10	10	10	8
200	10	10	10	10	10	10	10	10	10	10	10	10	10	10
100	10	10	10	10	10	10	10	10	10	10	10	10	10	10
50	10	10	10	10	10	10	10	10	10	10	10	10	10	10
25	10	10	10	10	10	10	10	10	10	10	10	10	10	10
Control	10	10	10	10	10	10	10	10	10	10	10	10	10	10
Females														
400	10	10	10	10	10	10	10	10	10	10	10	10	10	10
200	10	10	10	10	10	10	10	10	10	10	10	10	10	10
100	10	10	10	10	10	10	10	10	10	10	10	10	10	10
50	10	10	10	10	10	10	10	10	10	10	10	10	10	10
25	10	10	10	10	10	10	10	10	10	10	10	10	10	10
Control	10	10	10	10	10	10	10	10	10	10	10	10	10	10

000038

6694

APPENDIX D

WEEKLY DIET CONSUMPTION

APPENDIX D

AVERAGE INDIVIDUAL WEEKLY DIET CONSUMPTION (GRAMS)
DURING THE SUBCHRONIC TOXICITY STUDY OF NAPHTHALENE

Rats

Dose Group, mg/kg	Weeks of Study 1	2	3	4	5	6	7	8	9	10	11	12	13	Average Individual Daily Consumptio.
Males														
400	97.5	100.8	113.5	116.7	112.0	103.1	105.2	99.4	66.8	101.6	103.1	108.6	94.0	14.5
200	102.8	109.2	109.8	105.6	107.3	103.9	107.6	103.8	98.1	78.9	103.5	100.6	103.6	14.7
100	112.5	106.6	104.9	99.9	74.9	118.7	109.4	98.3	98.0	84.1	99.2	102.6	102.1	14.4
50	116.7	107.9	106.4	101.9	94.4	96.7	102.5	101.3	104.6	80.9	96.7	99.3	103.1	14.4
25	116.7	112.2	107.2	99.7	101.9	102.2	101.2	93.1	93.0	94.0	94.1	98.8	95.8	14.4
Control	117.0	109.8	106.4	101.2	98.8	98.5	100.5	98.7	96.4	89.6	96.1	99.4	93.2	14.3
Females														
400	59.3	61.3	66.5	76.0	68.6	66.0	65.8	67.9	80.5	48.7	57.9	58.1	51.8	9.1
200	64.0	67.0	70.8	68.5	67.5	68.9	65.3	66.4	74.1	58.2	57.6	66.1	66.5	9.5
100	70.7	70.3	68.1	61.4	66.1	63.2	67.1	67.7	70.8	57.5	58.2	63.5	84.3	9.5
50	73.3	67.5	67.7	62.3	62.9	65.8	61.3	81.4	75.5	52.7	56.4	65.4	62.0	9.4
25	71.3	69.3	61.5	69.0	63.9	56.6	58.5	68.5	73.1	57.4	62.0	62.5	59.2	9.2
Control	72.9	69.6	67.0	63.2	65.0	62.3	65.1	59.7	62.0	63.5	71.0	68.5	64.6	9.4

APPENDIX E

GROUP AVERAGE BODY WEIGHTS, WEEKLY INDIVIDUAL STARTING AND FINAL BODY WEIGHTS GRAPHS

APPENDIX E

AVERAGE BODY WEIGHTS (GRAMS) OF FISCHER RATS
IN THE SUBCHRONIC TOXICITY STUDY OF NAPHTHALENE

Dose Group, mg/kg	Weeks on Test 0	1	2	3	4	5	6	7	8	9	10	11	12	13	Differential Weight Gain, percent
Males															
400	190.3	196.6	207.3	220.3	222.3	242.1	245.3	244.7	244.8	231.8	236.7	240.1	256.8	250.6	-60.8
200	189.1	201.0	220.7	236.8	247.8	259.3	270.1	279.3	287.8	293.0	282.3	291.7	301.7	306.7	-23.6
100	188.5	208.5	229.9	243.0	257.1	245.4	274.5	286.7	297.4	303.9	304.4	312.0	325.2	333.4	- 5.8
50	193.2	221.1	239.6	257.2	269.8	278.2	288.0	303.2	312.6	323.1	317.7	329.7	343.1	351.2	2.7
25	192.8	220.0	241.0	258.0	269.0	281.4	293.7	304.9	315.1	318.6	326.3	335.5	347.3	353.4	4.4
Control	195.0	222.7	242.3	259.8	272.8	285.7	297.3	308.2	320.4	328.1	325.9	327.9	341.4	348.9	
Females															
400	137.6	136.6	144.2	147.6	157.9	162.9	165.0	165.5	167.6	168.0	164.5	162.1	166.9	156.7	-68.8
200	138.5	143.9	150.4	158.2	164.6	170.3	173.9	177.7	182.5	186.9	183.3	182.4	188.2	190.5	-15.0
100	143.9	152.9	160.7	164.9	170.0	173.5	177.7	181.5	186.6	191.6	189.4	190.1	193.7	197.2	-12.9
50	143.5	152.2	160.8	166.4	172.5	175.6	179.8	169.2	187.6	195.6	194.5	195.1	200.8	203.5	- 2.0
25	140.4	149.1	157.1	163.1	164.0	169.7	172.3	175.3	180.7	189.5	188.4	190.2	195.7	197.8	- 6.2
Control	142.2	153.2	159.8	171.0	172.7	175.6	181.1	184.5	187.7	189.0	191.5	194.4	200.3	203.4	

000042

INDIVIDUAL STARTING AND FINAL BODY WEIGHTS (GRAMS), AND PERCENT INCREASE, OF FISCHER 344 RATS DURING THE SUBCHRONIC TOXICITY STUDY OF NAPHTHALENE

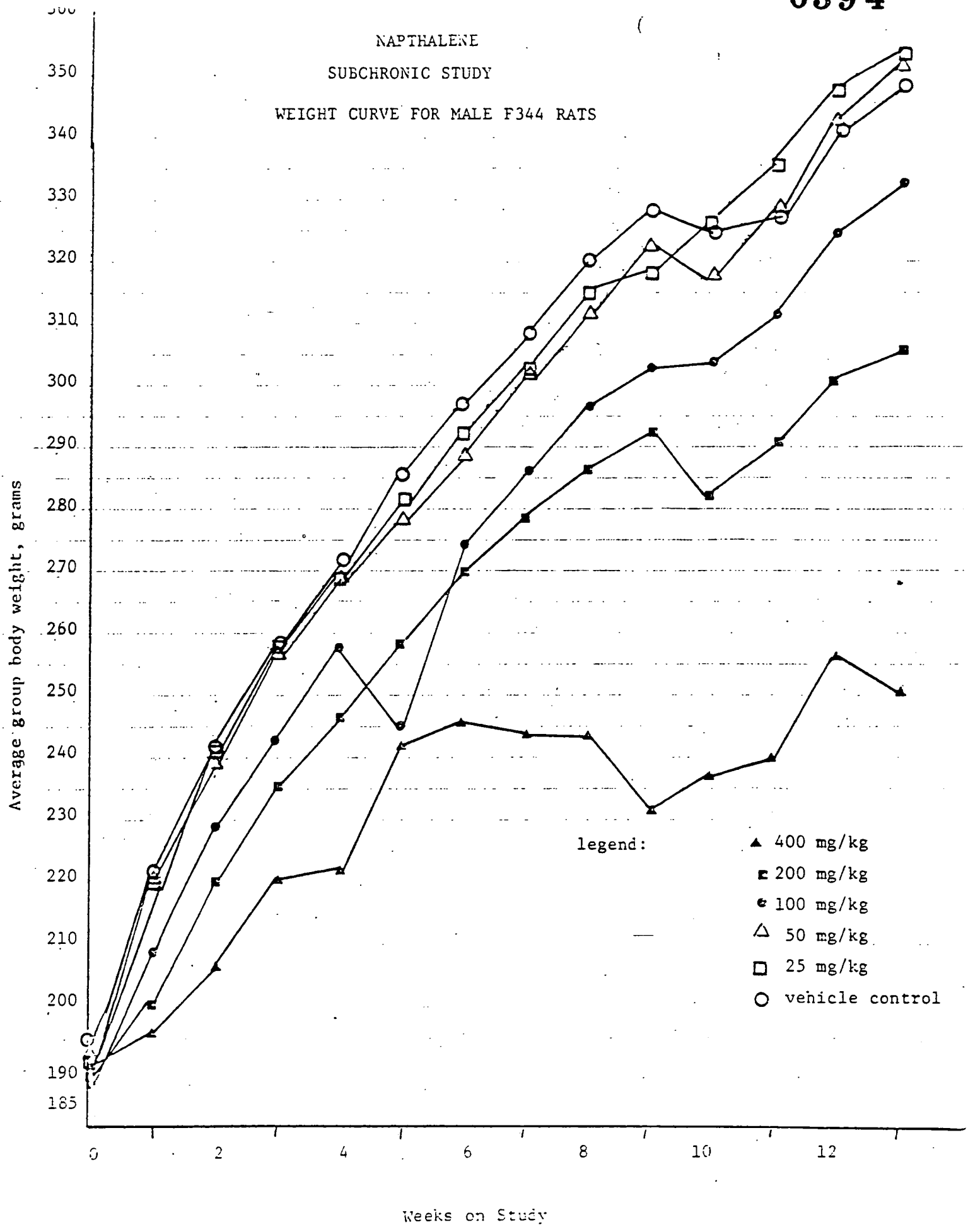
Dose Group, mg/kg	Animal Number	Day 0	Day 91	Percent Increase
		Males		
400	1	204	255	25
	2	197	289	47
	3	190	228	20
	4	166	--	--
	5	187	236	26
	6	202	266	32
	7	205	292	42
	8	185	272	47
	9	188	162	-14
	10	179	255	42
200	1	189	309	63
	2	173	285	65
	3	178	284	60
	4	210	333	59
	5	193	337	75
	6	172	287	67
	7	188	328	74
	8	182	291	60
	9	197	289	47
	10	209	324	55
100	1	177	308	74
	2	196	343	75
	3	210	368	75
	4	183	341	86
	5	185	334	81
	6	172	319	85
	7	201	341	70
	8	179	327	83
	9	185	315	70
	10	197	338	72
50	1	180	331	84
	2	207	354	71
	3	200	341	71
	4	213	402	89
	5	167	315	89
	6	209	365	75
	7	202	349	73
	8	200	350	75
	9	186	377	103
	10	168	328	95

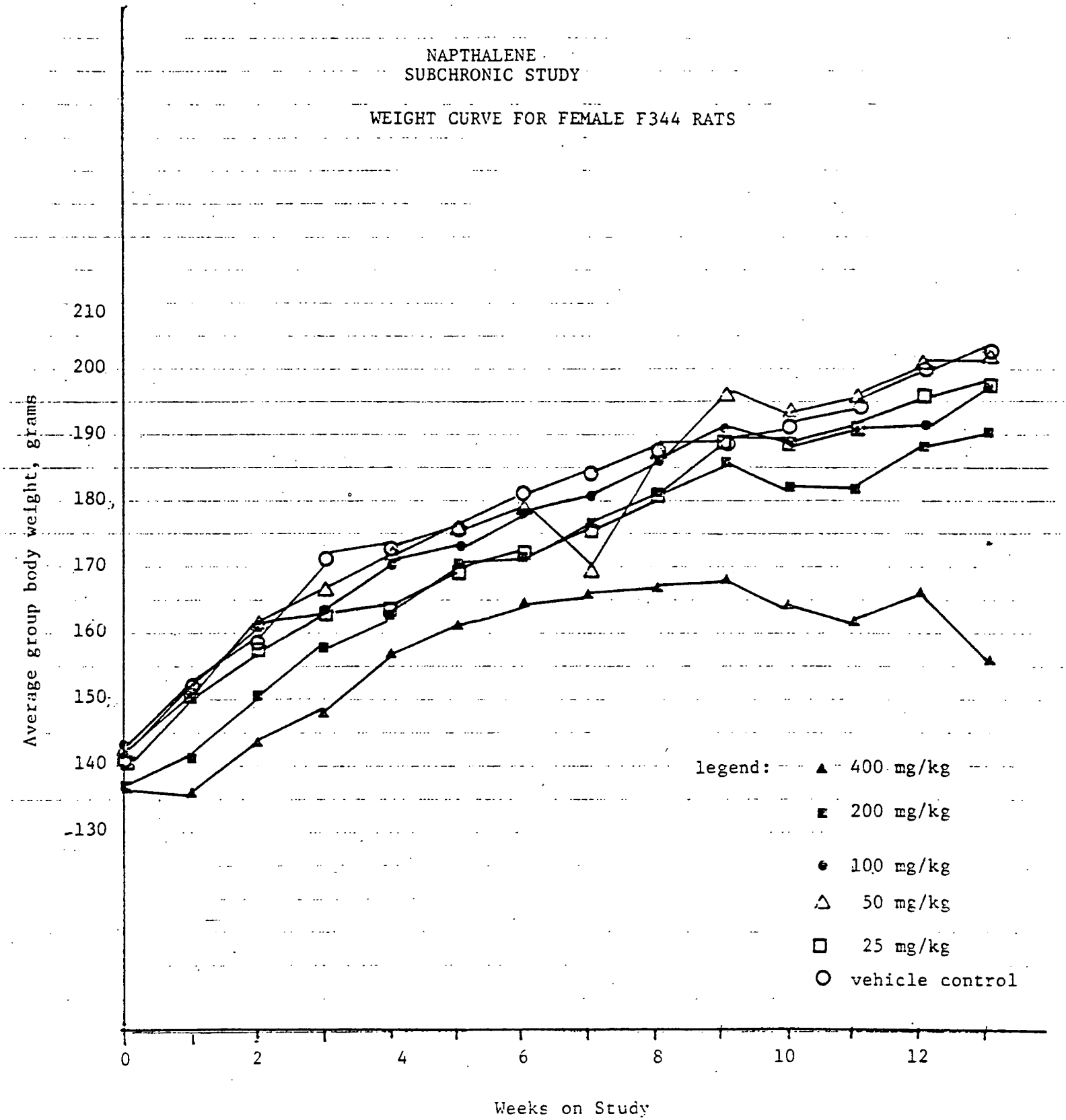
INDIVIDUAL STARTING AND FINAL BODY WEIGHTS (Continued)

Dose Group, mg/kg	Animal Number	Day 0	Day 91	Percent Increase
		Males (Continued)		
25	1	204	364	78
	2	181	365	102
	3	188	350	86
	4	188	327	74
	5	188	327	74
	6	197	344	75
	7	182	345	90
	8	205	380	85
	9	185	353	91
	10	210	379	80
Control	1	199	355	78
	2	186	323	74
	3	187	346	85
	4	196	366	87
	5	194	367	89
	6	208	360	73
	7	183	351	92
	8	191	336	76
	9	198	336	70
	10	208	349	68
		Females		
400	1	131	128	- 2
	2	134	162	20
	3	139	167	20
	4	140	184	31
	5	141	133	- 6
	6	138	167	21
	7	148	177	20
	8	134	165	23
	9	132	163	23
	10	139	121	-13
200	1	144	198	38
	2	145	207	43
	3	136	193	42
	4	140	185	32
	5	149	199	34
	6	131	174	33
	7	134	182	36
	8	131	187	43
	9	135	191	41
	10	140	189	35

INDIVIDUAL STARTING AND FINAL BODY WEIGHTS (Continued)

Dose Group, mg/kg	Animal Number	Day 0	Day 91	Percent Increase
		Females (Continued)		
100	1	154	201	31
	2	150	205	37
	3	151	203	34
	4	133	183	38
	5	135	189	40
	6	144	205	42
	7	145	188	30
	8	142	205	44
	9	144	203	41
	10	141	190	35
50	1	140	201	44
	2	132	184	39
	3	153	199	30
	4	138	208	51
	5	146	197	35
	6	152	210	38
	7	148	208	41
	8	146	226	55
	9	145	203	40
	10	135	199	47
25	1	141	188	33
	2	140	198	41
	3	137	195	42
	4	138	195	41
	5	143	203	42
	6	144	203	41
	7	140	202	44
	8	142	205	44
	9	132	188	42
	10	147	201	37
Control	1	153	217	42
	2	144	191	33
	3	147	210	43
	4	143	191	34
	5	139	196	41
	6	145	219	51
	7	142	218	54
	8	137	204	49
	9	135	191	41
	10	137	197	44


NAPTHALENE
SUBCHRONIC STUDY
WEIGHT CURVE FOR MALE F344 RATS
Average group body weight, grams
350
340
330
320
310
300
290
280
270
260
250
240
230
220
210
200
190
185
0
2
4
6
8
10
12
Weeks on Study
legend:
400 mg/kg
200 mg/kg
100 mg/kg
50 mg/kg
25 mg/kg
vehicle control


NAPTHALENE
SUBCHRONIC STUDY
WEIGHT CURVE FOR FEMALE F344 RATS
Average group body weight, grams
210
200
190
180
170
160
150
140
130
0
2
4
6
8
10
12
Weeks on Study
legend:
400 mg/kg
200 mg/kg
100 mg/kg
50 mg/kg
25 mg/kg
vehicle control

APPENDIX F

HEMATOLOGY TABLE

HEMATOLOGY TABLE

Naphthalene Subchronic Study

Rats

Dose, mg/kg	Statis-tics*	HgB	HCT	WBC	RBC	MCV	Bands	Segs	EOS	BASO	Lymph	Mono
						Males						
Control	$\bar{X}$	16.86	47.70	7.45	9.39	51.90	0	19.22	1.11	0	78.78	0.89
	SD(N)	0.63(10)	2.03(10)	1.05(10)	0.40(10)	0.57(10)	0(9)	6.16(9)	1.36(9)	0(9)	6.59(9)	1.69(9)
25	$\bar{X}$	17.30	49.80	8.64	9.32	54.60	0	16.90	0.80	0	82.20	0.10
	SD(N)	0.94(10)	1.93(10)	0.74(10)	0.45(10)	1.78(10)	0(10)	3.51(10)	0.42(10)	0(10)	3.29(10)	0.32(10)
50	$\bar{X}$	16.70	48.10	8.23	9.25	52.80	0	19.00	1.10	0	77.60	2.40
	SD(N)	0.47(10)	2.28(10)	0.68(10)	0.23(10)	1.69(10)	0(10)	4.47(10)	0.88(10)	0(10)	5.17(10)	2.17(10)
100	$\bar{X}$	16.84	46.70	7.61	9.24	51.6	0	20.0	1.11	0	78.0	0.89
	SD(N)	0.62(10)	1.89(10)	0.70(10)	0.35(10)	0.52(10)	0(9)	6.95(9)	1.27(9)	0(9)	7.71(9)	1.05(9)
200	$\bar{X}$	16.71	46.6	8.31	9.21	51.6	0	20.10	2.10	0	78.1	0.30
	SD(N)	0.66(10)	1.84(10)	0.75(10)	0.29(10)	0.52(10)	0(10)	5.09(10)	1.91(10)	0(10)	5.53(10)	0.65(10)
400	$\bar{X}$	15.48	44.75	8.9	8.60	53.12	0	37.29	1.14	0	59.00	2.57
	SD(N)	0.52(8)	1.91(8)	2.07(8)	0.37(8)	0.83(8)	0(7)	9.12(7)	1.86(7)	0(7)	9.09(7)	2.07(7)
						Females						
Control	$\bar{X}$	16.23	46.5	5.64	8.55	55.8	0	18.22	1.22	0	78.0	2.67
	SD(N)	0.51(10)	3.10(10)	0.70(10)	0.65(10)	1.23(10)	0(9)	4.58(9)	1.20(9)	0(9)	5.29(9)	1.58(9)
25	$\bar{X}$	16.46	42.56	6.44	8.10	53.33	0	18.67	0.44	0	79.33	1.56
	SD(N)	0.40(9)	1.51(9)	0.45(9)	0.25(9)	0.50(9)	0(9)	6.00(9)	0.73(9)	0(9)	6.86(9)	1.81(9)
50	$\bar{X}$	16.53	44.30	8.76	8.31	54.30	0	16.10	1.50	0	81.70	0.70
	SD(N)	0.53(10)	1.64(10)	1.06(10)	0.32(10)	0.48(10)	0(10)	4.51(10)	1.08(10)	0(10)	3.92(10)	1.06(10)
100	$\bar{X}$	16.76	45.80	7.78	8.65	54.00	0	19.00	1.44	0	77.22	2.33
	SD(N)	0.89(10)	1.93(10)	0.85(10)	0.43(10)	0.47(10)	0(9)	3.08(9)	1.33(9)	0(9)	3.99(9)	1.41(9)
200	$\bar{X}$	16.40	43.00	7.58	8.38	52.90	0	19.10	2.00	0	78.67	0.17
	SD(N)	0.54(10)	2.05(10)	0.98(10)	0.47(10)	0.74(10)	0(6)	4.86(6)	1.67(6)	0(6)	5.16(6)	0.41(6)
400	$\bar{X}$	15.27	43.50	7.37	8.19	54.10	0	25.00	1.00	0	74.00	0
	SD(N)	0.93(10)	2.80(10)	1.12(10)	0.59(10)	1.20(10)	0(10)	9.56(10)	1.05(10)	0(10)	9.44(10)	0(10)

* $\bar{X}$ = Mean; SD = Standard Deviation; (N) = Sample Number

APPENDIX G

PURINA LAB CHOW FEED TAG

LABORATORY CHOW

50

1005

RALSTON PURINA CO. ST. LOUIS MO

1005

PURINA®
LABORATORY CHOW®

RALSTON PURINA CO.
Gen. Offices, St. Louis, Mo. 63188

GUARANTEED ANALYSIS

Crude protein not less than	23.0%
Crude fat not less than	4.0%
Crude fiber not more than	5.0%
Ash not more than	8.0%
Added minerals not more than	2.5%

INGREDIENTS

Ground extracted corn, soybean meal, ground oat groats, dried beet pulp, wheat germ meal, fish meal, condensed alfalfa meal, dried cane molasses, meat and bone meal, brewers' dried yeast, wheat middlings, animal fat preserved with BHA, calcium carbonate, dicalcium phosphate, salt, calcium iodate, animal liver meal, vitamin B_{12} supplement, methionine hydroxy analogue calcium, calcium pantothenate, choline chloride, folic acid, ascorbyl monophosphate, inositol, niacin, pyridoxine hydrochloride, ferrous sulfate, vitamin A supplement, d activated animal sterol, vitamin E supplement, iron sulfate, iron oxide, manganous oxide, cobalt carbonate, copper oxide, zinc oxide.

F-5001-

50 POUNDS NET WEIGHT