NCTR Technical Report

for

Experiment No. 376
NTP Experiment No. 5010-02

FINAL REPORT

DOXYLAMINE
90 DAY SUBCHRONIC STUDY IN B6C3F1 MICE

National Center for Toxicological Research
Jefferson, Arkansas 72079

September 1982

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90 DAY SUBCHRONIC STUDY REPORT ON

DOXYLAMINE

IN B6C3F1 MICE

NATIONAL TOXICOLOGY PROGRAM

NCTR EXPERIMENT NO. 376

NTP EXPERIMENT NO. 5010-02

NATIONAL CENTER FOR TOXICOLOGICAL RESEARCH

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ABSTRACT

Doxylamine is a drug used as an antihistamine in allergies, rhinitis and skin conditions and has sedative and mild local anesthetic properties. Its use as an antihistamine has been minor but doxylamine has been used extensively as a sleep inducing agent. Doxylamine was selected for testing because of its widespread use and its chemical similarity to methylpyrilene, an antihistamine which has been found to be carcinogenic in rats.

A 14-day repeated-dose subchronic dosed-feed study was conducted on doxylamine. Based on the effects of the chemical on body weight gain and histopathological exams, dose levels of 0, 80, 162, 325, 750 and 1500 ppm were selected for this 90-day subchronic study in mice.

Dosed feed was available to the animals throughout the study. There were no chemical related deaths and all of the mice lived until the terminal sacrifice at the end of the study. Clinical observations during the study were not remarkable. Except for a few scattered instances of scratches and sores, the only significant clinical observations were skinny appearances which correlated with reduced body weight gain. When body weight gain was compared to gain of controls, there was a reduced weight gain in all but the lowest dose level in males and in every dose group in females. Weight gain was reduced up to 44% in males and 40% in females. These values, however, reflect differences due to small weight differences (2-4 grams) and are difficult to interpret with precision. A better determinant of chemical effect may be the comparison of final body weights. In this comparison, the same dose levels were effective but the difference was more modest with the highest dose level (1500 ppm) producing a reduction of 8.0% and 10.3%, in males and females, respectively, compared to controls. Considering the erratic nature of mouse body weight data, there was a minimal effect even at the highest dose level.

There were no significant abnormal observations made at necropsy in either male or female mice. Microscopic lesions which were chemical related were found in the liver only. The lesions consisted of cell cytomegaly or karyomegaly which varied from mild to severe, in low to high dose levels, respectively. Only females in the lowest dose group did not have treatment related lesions. A mild degree of liver necrosis was approximately constant irrespective of dose. It was extensive in only three animals, one in the highest dose group and two in lower dose groups.

Serum levels of the hepatic enzyme ALT was elevated by treatment with doxylamine in both males and females while AST levels were elevated in females only.

Several organ weights appeared to be affected by doxylamine administration including, brain, liver, lung, kidney, testis, and thymus. The only two, however, that appeared consistent when either organ/body weight or organ/brain weight ratios were compared were increased liver weights and decreased kidney.

All the significant observations are summarized in the attached Table 1.

TABLE 1

SUMMARY OF SIGNIFICANT TOXICOLOGICAL PARAMETERS FOR MALE B6C3F1 MICE IN 90-DAY SUBCHRONIC STUDY ON DOXYLAMINE

Dose (% in feed)	Mortality	Weight Gain in Grams (% Difference) from controls)	Significant Clinical Observations	Significant Gross Observations	Significant Pathological Observations (incidence)
Control	0/12	9 (--)	Alopecia(2/12)	0/12	Lung-hemorrhage (1/12) Kidney-cytoplasmic vacuolization (1/12) Liver-vacuolated cell foci (1/12) Preputial glands-ectasia (3/6)
0.0080	0/12	8.9 (-1.9)	0/6	0/12	Liver-karyomegaly (1/12) -cytomegaly (4/12) -necrosis (4/12) -cytoplasmic vacuolization (6/12) -acute inflammation (1/12) -chronic inflammation (1/12) -calcification (2/12) -granulomatous inflammation (1/12)
0.0162	0/12	4.6 (-48.9)	Sores-anal (1/12) Scratches body (1/12) Scratches anal (1/12)	0/12	Liver-vacuolated cell foci (1/12) -karyomegaly (6/12) -cytomegaly (9/12) -cytoplasmic vacuolization (9/12)
0.0325	0/12	5.6 (-37.8)	Rough hair (4/12) Scratches (3/12)	0/12	Liver-karyomegaly (7/12) -cytomegaly (11/12) -nuclear inclusion (1/12) -necrosis (3/12) -cytoplasmic vacuolization (5/12) -lymphocytic accumualtion (1/12) -pigmentation (1/12)

TABLE 1 (continued)

SUMMARY OF SIGNIFICANT TOXICOLOGICAL PARAMETERS FOR MALE B6C3F1 MICE
IN 90-DAY SUBCHRONIC STUDY ON DOXYLAMINE

Dose (% in feed)	Mortality	Weight Gain in Grams (% Difference from controls)	Significant Clinical Observations	Significant Gross Observations	Significant Pathological Observations (incidence)
0.0750	0/12	7.5 (-17.2)	Alopecia(1/12)	0/12	Liver-karyomegaly (12/12) -cytomegaly (12/12) -nuclear inclusion (3/12) -necrosis (3/12) -cytoplasmic vacuolization(10/12) Preputial gland-ectasia (1/1)
0.1500*	0/12	5.0 (-44.8)	Scratches(1/12)	0/12	Lung-hemorrhage (1/12) Liver-karyomegaly (11/12) -cytoplasmic alteration (1/12) -cytomegaly (9/12) -necrosis (11/12) Kidney-tubular dilatation (1/12) Spleen-lymphoid hyperplasia (1/12) -erythropoiesis (1/12) Parotid gland-necrosis (1/12) Preputial gland-ectasia (4/5)

*Significant Clinial Pathological Observation - ALT & AST elevated at this dose level.

TABLE 1 (continued)

SUMMARY OF SIGNIFICANT TOXICOLOGICAL PARAMETERS FOR FEMALE B6C3F1 MICE
IN 90-DAY SUBCHRONIC STUDY ON DOXYLAMINE

Dose (% in feed)	Mortality	Weight Gain in Grams (% Difference from controls)	Significant Clinical Observations	Significant Gross Observations	Significant Pathological Observations (incidence)
Control	0/12	6.5 (-)	None	None	Lung-hemorrhage (4/12) Liver-vacuolated cell foci (1/12) -necrosis (2/12) Pancreas-islet cell hyperplasia (1/12)
0.0080*	0/12	6.0 (-8.5)	Skinny (1/12)	None	Liver-necrosis (3/12) -lymphocytic accumulation (1/12) -cytoplasmic vacuolization (1/12)
0.0162	0/12	6.2 (-4.9)	None	None	Liver-karyomegaly (1/12) -cytomegaly (1/12) -necrosis (3/12) -cytoplasmic vacuolization (6/12)
0.0325	0/12	5.3 (-19.4)	Skinny (2/12)	None	Liver-karyomegaly (4/12) -cytomegaly (6/12) -acute inflammation (2/12) -cytoplasmic vacuolization (2/12)
0.750	0/12	5.1 (-21.5)	Skinny (1/12)	None	Liver-karyomegaly (8/12) -cytomegaly (9/12) -necrosis (4/12) -acute inflammation (1/12) -cytoplasmic vacuolization (9/12)

TABLE 1 (continued)

SUMMARY OF SIGNIFICANT TOXICOLOGICAL PARAMETERS FOR FEMALE B6C3F1 MICE
IN 90-DAY SUBCHRONIC STUDY ON DOXYLAMINE

Dose (% in feed)	Mortality	Weight Gain in Grams (% Difference from controls)	Significant Clinical Observations	Significant Gross Observations	Significant Pathological Observations (incidence)
0.1500	0/12	3.9 (-40.0)	Skinny (3/12)	0/12	Lung-chronic inflammation (1/12) Liver-acidic cell foci (2/12) -karyomegaly (11/12) -cytomegaly (11/12) -cytoplasmic inclusion (1/12) -necrosis (12/12) Spleen-erythropoiesis (3/12) Parotid gland-necrosis (1/12)

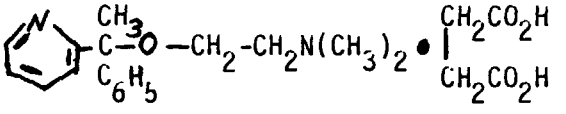
*Significant Clinical Pathological Observations - AST elevated at this dose level.

I. INTRODUCTION

Results of the doxylamine 14-day subchronic study did not demonstrate significant effects of the chemical at the highest dose level used (2000 ppm). However, since focal hepatic necrosis was observed in mice at the two highest dose levels, and since these were considered to be life threatening, dose levels of 0, 80, 162, 325, 750 and 1500 ppm were selected for this study. The purpose of this study was to determine the maximum tolerated dose (MTD) and to further identify possible target organs.

II. MATERIALS AND METHODS

A. Chemistry



Doxylamine Succinate (N,N-dimethyl-2-[1-phenyl-1-(2-pyridyl)-ethoxy]ethane, succinate)

Reference Sample: A reference sample of doxylamine succinate (10g; Lot #001111) was obtained from J.T. Baker Chemical Co., Vicks Health Care Division. The material obtained was a white solid and contained no detectable impurities. Methods of analysis with purity and chemical characterizations are presented in Appendix A.

Bulk Sample: A bulk sample of doxylamine succinate (12 kg, Lot #L0536-1) was obtained from Richardson-Merrell, Inc., Cincinnati, Ohio. The material received was a white solid and was found to be essentially 100% pure. The methods of analysis with purity and chemical characterizations are presented in Appendix A.

Stability Studies: The stability of doxylamine succinate mixed in animal feed was determined both under conditions of feed utilization (open feeder) and feed storage (sealed in the dark). Both the methods for determining stability as well as the results of the studies are given in Appendix A. Although an analysis was not performed on the highest dose level at the 36 day time point in the short-term study (open feed), it was deduced from the stability of the low dose as well as stability of the high dose for shorter times, that the compound is stable in open containers for up to 36 days. The results also indicated that doxylamine succinate is stable for up to 16 weeks under conditions used for dosed-feed storage. It was concluded that the test chemical is stable in the animal meal for sufficient time to allow administration of the test chemical to test animals in feed.

Storage Conditions: The bulk sample of doxylamine succinate was stored in its shipping container (fiberboard lined with polyethylene) in the dark at ambient temperature (15°-25°C).

Dosing Preparation and Analysis: A 10% stock solution of doxylamine succinate was prepared in 95% ethanol. Appropriate aliquots were added to 20 kg batches of ground Purina 5010M (meal) in 2-cubic foot Marion Mixer while being mixed. The stock solution was introduced under pressure through a spraying device to insure uniform mixing. After 5 to 15 minutes mixing, a vacuum of 12" Hg was applied and the temperature was maintained at 120°F by means of a hot water jacket. Mixing was continued for 30 minutes after the temperature reached 120°F. The vacuum was then released and the mixer was cooled by circulating tap water. The cooled feed was then mixed an additional 10 minutes. The mixed feed was stored in stainless steel milk cans and labeled appropriately. Samples were taken from selected batches for chemical analysis and dose verification. The feed was then transferred to specially constructed, sterile paper boxes (approximately 150 g feed/box) which fit into the mouse feeders. Each box was labeled with the batch number and dose code. The boxes of feed were sealed in stainless steel containers for transport to and sterilization into the barrier animal room.

After mixing a batch of dosed feed, the mixer was then cleaned and the next higher dose level of feed was mixed. If a lower dose level or a different chemical were to be mixed next, the mixer was thoroughly cleaned with appropriate solvents. Standard Operating Procedures (SOP's) for the mixing, packaging and storage of feed and for the operation and clean-up of equipment are on permanent file in the NCTR Archives.

Dosed feed was prepared using the folllowing dilution chart:

1. Stock Solution
   A. 2000 ml batch
      -288 g doxylamine succinate*
      -dilute to 2 l with 95% ethanol
   B. 4000 ml batch
      -576 g doxylamine succinate*
      -dilute to 4 l with 95% ethanol

   *Dose level was based on the free anime and not the succinate salt of the amine.

2. Dosed Feed

Target ppm	Stock Conc.	kg Feed	ml Stock	ml Ethanol
80	10%	20	16.0	1984
162	10%	20	32.0	1968
325	10%	20	65.0	1935
750	10%	20	150.0	1850
1500	10%	20	300.0	1700

Vehicle: The doxylamine succinate was added to the feed as a solution in 95% ethanol. No analysis of the ethanol was performed, but USP grade ethanol was used.

Personnel Protection: Doxylamine has been used widely as an antihistamine and mild sedative. The normal therapeutic dose for humans is 100 to 200 mg/kg, usually divided into four doses. Employees were unlikely to be exposed to amounts anywhere approaching therapeutic dose levels, however, instructions given in the NCTR Safety Manual, Section VI.B and VI.E were followed in this study.

Doxylamine was used on the open bench when less than one gram of the chemical was being used. Due to the improbability of personnel receiving toxic doses, it was not necessary to document the open-handling provision for animal caretakers working in animals rooms or in support areas where cages, etc. are cleaned.

When being moved from one place to another, doxylamine was contained as described in the NCTR Safety Manual, Section VI.C.

Employees conducting research with doxylamine were provided protective clothing in compliance with the NCTR Safety Manual, Section VI.D. To prevent skin contact, employees wore impervious gloves and/or face shield where appropriate in compliance with the NCTR Safety Manual. Where dust or vapors and/or odors of doxylamine were present, a respirator providing protection equivalent to 3M8710 or 3M9910 was provided.

Decontamination of laboratories and equipment was by the procedures given in the NCTR Safety Manual, Section VI.G. The workplace was monitored periodically and analyzed by methods similar to those described for feed analysis.

B. Animals

Source: B6C3F1 mice, male and female, 37 days of age were used in this study. Animals within the weight range 14.2g to 25.6g for males and 12.3g to 21.8g for females were allocated. The original breeding stock was obtained from National Institute of Health colonies and were produced and maintained under Specific Pathogen Free (SPF) conditions. Female C56Bl/6N and male C3H/HeN MTV- mice were crossed to produce the B6C3F1 hybrid animals.

Quarantine: Since the experimental animals were produced on-site under SPF conditions with continuous microbiological surveillance, no quarantine period was necessary.

Disease and parasite exam: Microbiological surveillance of the production colony was on a continued basis. As of April 1, 1982, 21 female mice of the C57Bl/6N strain and 21 male mice of the C3H/HeN MTV- strain were evaluated for primary potential pathogens. Two of the C57Bl/6N parents were positive for Reo-3 virus with low titers of 1:20 and 1:40. One parent of the C3H/HeN MTV- strain was positive for Encephalomyelitus virus (GDVII) with a low titer of 1:40. No other potential microbiolo-

gical pathogens were identified or isolated. Environmental samples from all the breeder rooms were taken on a weekly basis and consisted of feed, cage water, bedding and waste, room air samples, and room and equipment swab samples. All samples were negative for potential pathogenic organisms or did not exceed microbial standards for non-pathogenic organisms. In the experimental animal rooms, composite bedding and waste samples, room air samples, and cage and water samples were sampled from each room on a monthly basis. Approximately two feed samples per experiment were sampled on a weekly basis. Room swab samples were evaluated prior to initiation of the experiment. In this study five feed samples were found to contain excessive bacterial and/or mold contamination and all five were discarded.

Allocation and Randomization: Seventy-two male and 72 female mice were divided into 6 dose groups (5 dose levels plus 1 control group) with 12 mice per group for each sex. The mice were allocated to cages randomly on a weight basis. At the time of allocation, the available mice were weighed and arranged in order from lightest to heaviest. The lightest and heaviest were then discarded in alternating sequence until 72 mice remained. The allocation then proceeded such that the 12 lightest anidmals were assigned at random with two animals going to each dose group. The procedure was then repeated with the next lightest 12 mice, etc., until all 72 had been allocated. Each cage was randomly assigned to one rack for each sex. Details of random allocation of animals are presented in Appendix C.

Animals were identified during the experiment by rack and cage number and by ear clip. Each of the four mice per cage were ear clipped "Both", "None", "Left", or "Right". At the time of death of an animal, each animal was assigned a CID number (Carcass Identification) on the EIS terminal or a manual EIS form was completed. When a mouse was removed for a scheduled sacrifice, the EIS terminal was used and the CID was assigned but no EDCS form was required. CID numbers for the mice of this ot this study were preassigned as 37600145 through 37600288.

C. Animal Maintenance

This study was housed in room 151 of Building 5 (A Barrier). Rats, which were also administered doxylamine succinate, were housed in the same room with mice. Mice were housed four per cage in polycarbonate cages that measure 10 1/2" x 6 1/2 " x 5". Hardwood chip bedding was used. Cages were placed on movable stainless steel racks which held 18 cages arranged in 6 columns of 3 cages each. The mice received filtered water from the potable water system which was derived from the well water system. Analysis of the well water is presented in Appendix B. The mice were maintained on control or dosed Purina 5010 M ground meal laboratory chow.

The mice were maintained in a barrier room which was well ventilated and temperature and humidity controlled. Outside air

supplied to the room was passed through a high-efficiency particulate air (HEPA) filter and not recirculated. There were about 17 changes of air per hour with static room air pressure maintained slightly positive to the outside environment. Relative humidity was maintained at set-point of 50% (alarm range: 40-60%) and the set-point for room temperature was 72% (alarm range 70°-75°F). The room was on a 12 hour light/12 hour dark cycle with fluorescent lighting maintained at 100 foot candles at 3 feet above floor level. All materials entering the room were sterilized. Personnel used clothing and sanitation procedures to meet SPF barrier specifications. Room temperature and humidity were recorded throughout the study. Temperature was recorded 8746 times during the study with 9 recordings exceeding the specifications and none being too low. Humidity readings were made 8711 times with 25 being higher than the specifications. The temperature and humidity readings that were out of the targeted range are presented in Appendix A.

Products used in the maintenance of animals are described in Appendix B.

D. Study Design

The details of the design used in this study are given in the protocol (Appendix C). The operational protocols, designated as Standard Operating Procedures (SOP) are on permanent file in the NCTR Archives. There were no major revisions to the protocol but the minor changes made are presented in Appendix C.

B6C3F1 mice, male and female, were administered doxylamine succinate at dose levels of 0 (control diet) 80, 162, 325, 750 and 1500 ppm. The dose was calculated and prepared on the basis of the free amine and not the succinate. Twelve animals of each sex were treated at each dose level. The chemical was administered in the feed ad libitum for 90 days. All the mice were placed on dosed feed on July 15, 1981 and 36 animals per day were removed from the test diets on October 13-16, 1981. The animals were removed from the diets in the afternoon and fasted overnight until sacrifice the next morning. They were provided water ad libitum at all times.

E. Data Storage

The location of all data and experimental specimens are identified in the Location Directory, Appendix C.

III. RESULTS

A. Body Weights

Individual body weights are presented in Appendix C. Terminal weights are summarized in Appendix C in the table "Group Mean Terminal Weights" and "Organ/Terminal Weight Ratios". Terminal weights also are presented in Results under "E. Necropsy Data" with their difference from controls presented as percentage.

Body weights are presented graphically in the following Figures 1 and 2. There was a poor dose-response in effect on weight gain. When terminal weights had been corrected for differences in initial weights, there was a reduction in terminal weights of 8% to 14% over the dose-range of 162 ppm to 1500 ppm in males. Females exhibited a similar effect with a decreased terminal weight of 5% at 325 ppm to 10% at 1500 ppm. When weight gain was compared, there was a decrease at every treatment level ranging from 2% to 49%, with some intermediate doses having the greatest decrease.

B. Food Consumption

Food consumption and amount of chemical administered data are presented in Tables 2 and 3 and Figures 3 and 4. There was little or no effect of the doxylamine on food consumption at any dose level except the very highest (1500 ppm). The highest dose group appears to have consumed slightly more food than the other doses or controls in nine out of the thirteen weeks. This effect was slight, however, and had little effect on average chemical consumed.

C. Survival

All animals, both male and female, survived until the scheduled terminal sacrifice at the end of the 90 day study.

D. Clinical Observations

There were few treatment-related clinical observations during the course of the study. Several of the male rats were observed to have scratches at 162, 325, and 750 ppm but only 1/12 at the highest dose level of 1500 ppm. The only other sign that appeared to be treatment related was a skinny appearance which was found in females only. Details of the clinical observations including specific animal identification and day of first observation are presented in Appendix C.

E. Necropsy Data

Necropsies were performed on all animals; however, there were no gross lesions observed. Final body weights are shown in Table 4.

F. Hematology and Clinical Chemistry

No hematology data were obtained in this study. Aspartate aminotransferase (AST or SGOT) and alanine aminotransferase (ALT or SGPT) levels were determined. Because animals of different dose groups were sacrificed on different days, the effect of day differences could not be factored from dose effects. Consequently, the results, presented in Appendix C, combine the two variables. The statistical analysis results are presented in Table 5. There was a treatment effect on AST in females and on ALT on both males and females.

FIGURE 1

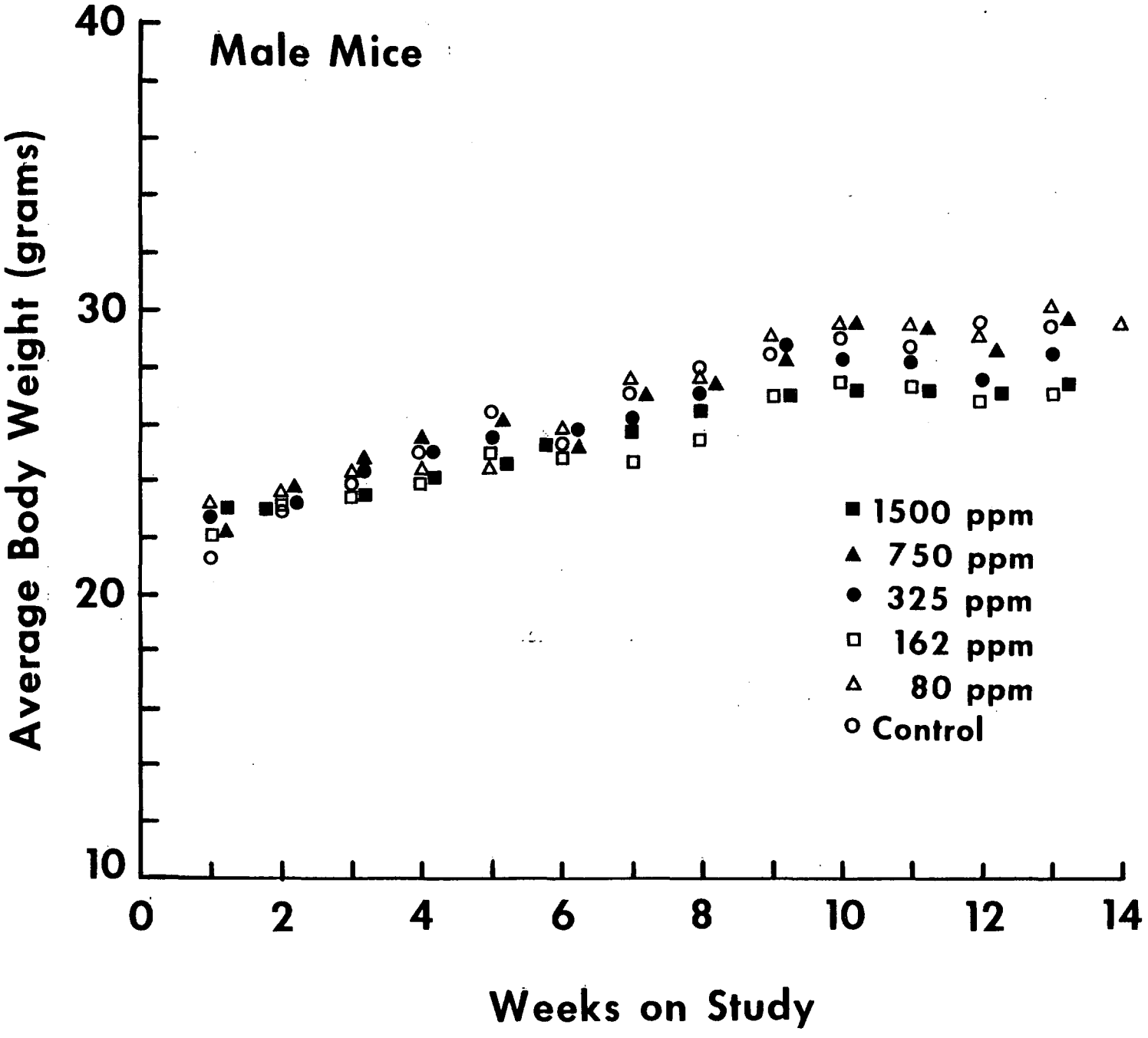

Male Mice
Average Body Weight (grams)
40
30
20
10
0
2
4
6
8
10
12
14
Weeks on Study
1500 ppm
750 ppm
325 ppm
162 ppm
80 ppm
Control

FIGURE 2

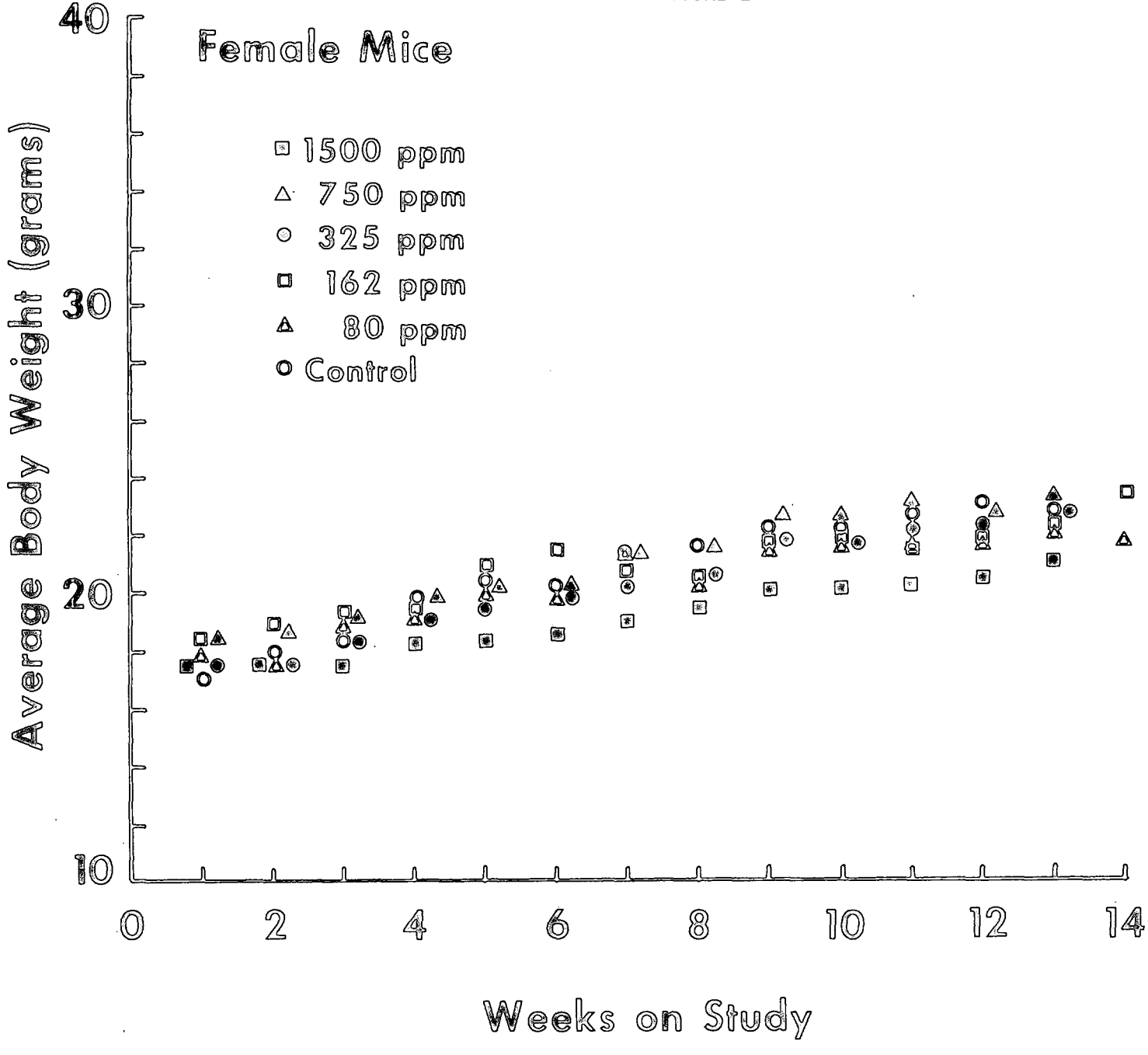

Female Mice
1500 ppm
750 ppm
325 ppm
162 ppm
80 ppm
Control
Average Body Weight (grams)
40
30
20
10
0
2
4
6
8
10
12
14
Weeks on Study

TABLE 2

AVERAGE WEEKLY FOOD AND CHEMICAL CONSUMPTION BY GROUP
NTP EXPERIMENT 05010 TEST 02
MALE MICE

DOSE	WEEK 2		3		4		5		6		7		8	
(% IN FEED)	FOOD	CHEM	FOOD	CHEM	FOOD	CHEM	FOOD	CHEM	FOOD	CHEM	FOOD	CHEM	FOOD	CHEM
0	153.2	0.0	142.8	0.0	142.3	0.0	130.7	0.0	139.9	0.0	146.0	0.0	136.0	0.0
0.008	147.0	11.8	142.3	11.4	129.5	10.4	142.7	11.4	140.1	11.2	140.8	11.3	127.9	10.2
0.0162	165.2	26.8	147.3	23.9	143.6	23.3	138.2	22.4	145.0	23.5	145.2	23.5	151.6	24.6
0.0325	147.3	47.9	156.1	50.7	143.2	46.5	145.9	47.4	147.9	48.1	147.2	47.8	141.9	46.1
0.075	153.9	115.4	148.3	111.2	134.6	100.9	135.8	101.8	138.1	103.6	144.0	108.0	138.0	103.5
0.15	141.8	212.7	166.5	249.7	153.1	229.6	157.8	236.7	152.3	228.4	151.0	226.5	149.3	224.0

DOSE	WEEK 9		10		11		12		13		14		AVERAGE FOR STUDY	
(% IN FEED)	FOOD	CHEM	FOOD	CHEM	FOOD	CHEM	FOOD	CHEM	FOOD	CHEM	FOOD	CHEM	FOOD	CHEM
0	135.8	0.0	130.2	0.0	135.1	0.0	136.2	0.0	136.2	0.0			138.7	0.0
0.008	136.6	10.9	126.3	10.1	124.9	10.0	116.3	9.3	135.0	10.8	169.8	13.6	136.9	10.9
0.0162	149.8	24.3	141.3	22.9	139.9	22.7	135.1	21.9	144.7	23.4	135.9	22.0	144.8	23.5
0.0325	148.1	48.1	133.2	43.3	132.4	43.0	129.8	42.2	147.6	48.0	135.1	43.9	142.7	46.4
0.075	136.6	102.5	129.0	96.7	124.3	93.2	126.4	94.8	130.8	98.1			136.6	102.5
0.15	150.9	226.4	148.1	222.2	137.6	206.4	137.3	205.9	152.7	229.0			149.9	224.8

FOOD - TOTAL GRAMS CONSUMED/TOTAL WGT KILOGRAMS PER DAY
CHEM - MILLIGRAMS CHEM CONSUMED/KG BW PER DAY
AVERAGE FOR STUDY FOOD - MEAN FOOD CONSUMED DURING STUDY
CHEM - MEAN CHEMICAL CONSUMED DURING STUDY
CHEMICAL ANALYSIS INDICATES PURITY OF 100.0%

TABLE 3

AVERAGE WEEKLY FOOD AND CHEMICAL CONSUMPTION BY GROUP
NTP EXPERIMENT 05010 TEST 02
FEMALE MICE

DOSE	WEEK 2		3		4		5		6		7		8	
(% IN FEED)	FOOD	CHEM	FOOD	CHEM	FOOD	CHEM	FOOD	CHEM	FOOD	CHEM	FOOD	CHEM	FOOD	CHEM
0	182.7	0.0	187.2	0.0	182.4	0.0	162.8	0.0	167.5	0.0	179.9	0.0	155.6	0.0
0.008	176.7	14.1	185.3	14.8	165.4	13.2	170.7	13.7	170.2	13.6	165.6	13.2	155.4	12.4
0.0162	186.3	30.2	182.9	29.6	175.9	28.5	169.3	27.4	169.6	27.5	167.0	27.1	174.7	28.3
0.0325	193.2	62.8	197.4	64.2	187.3	60.9	179.0	58.2	187.0	60.8	174.4	56.7	171.3	55.7
0.075	173.6	130.2	179.6	134.7	175.0	131.3	158.3	118.7	163.5	122.6	166.2	124.6	161.9	121.4
0.15	191.3	286.9	218.7	328.1	196.7	295.0	200.7	301.1	198.8	298.2	193.1	289.7	180.0	270.1

DOSE	WEEK 9		10		11		12		13		14		AVERAGE FOR STUDY	
(% IN FEED)	FOOD	CHEM	FOOD	CHEM	FOOD	CHEM	FOOD	CHEM	FOOD	CHEM	FOOD	CHEM	FOOD	CHEM
0	164.1	0.0	155.9	0.0	170.9	0.0	149.9	0.0	158.3	0.0			168.1	0.0
0.008	169.2	13.5	163.6	13.1	157.8	12.6	153.9	12.3	164.3	13.1	208.8	16.7	169.7	13.6
0.0162	180.0	29.2	169.2	27.4	171.7	27.8	161.4	26.1	172.4	27.9	219.5	35.6	176.9	28.7
0.0325	174.8	56.8	165.6	53.8	163.8	53.2	152.4	49.5	164.4	53.4	154.1	50.1	174.2	56.6
0.075	166.7	125.1	151.6	113.7	159.7	119.8	152.4	114.3	154.2	115.7			163.6	122.7
0.15	191.0	286.5	195.4	293.2	192.0	288.0	172.1	258.2	182.7	274.1			192.7	289.1

FOOD - TOTAL GRAMS CONSUMED/TOTAL WGT KILOGRAMS PER DAY
CHEM - MILLIGRAMS CHEM CONSUMED/KG BW PER DAY
AVERAGE FOR STUDY FOOD - MEAN FOOD CONSUMED DURING STUDY
CHEM - MEAN CHEMICAL CONSUMED DURING STUDY
CHEMICAL ANALYSIS INDICATES PURITY OF 100.0%

FIGURE 3

AVERAGE FOOD, WATER, WEIGHT, AND WEIGHT GAIN (GRAMS) BY TREATMENT GROUP
DOXYLAMINE

NTP
EXPERIMENT: 05010 TEST: 02

TEST TYPE: SUBCHRON 90-DAY
PI: DR. C. D. JACKSON, NCTR

ROUTE: ORAL FOOD

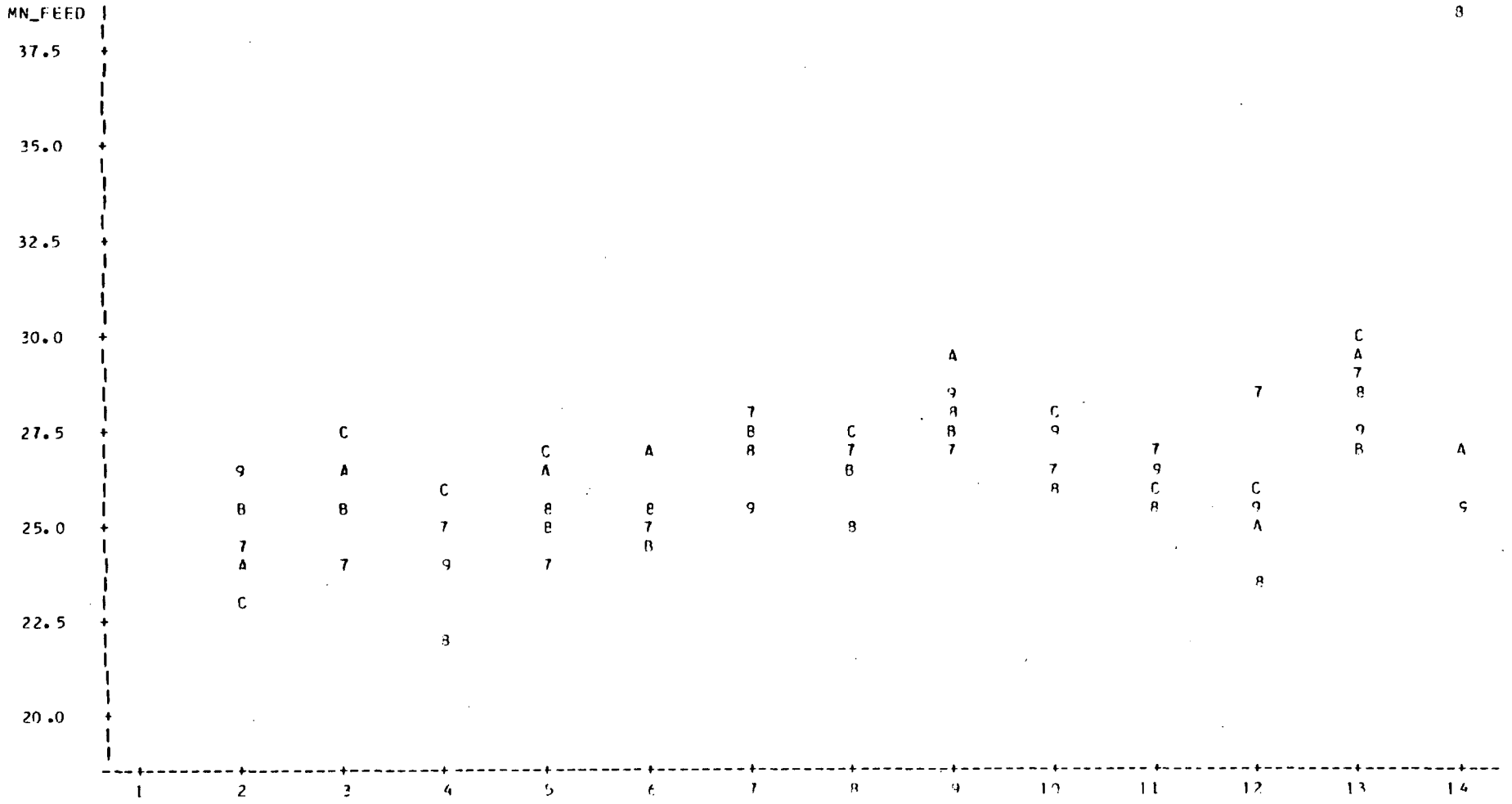
REPORT: EIS11-12
DATE: 09/22/82
TIME: 14:33:37

NTP: IC195F
CAS: 000469216
CONT:

*
PLOT OF AVERAGE FOOD CONSUMPTION (GRAMS/ANIMAL/WEEK) BY DOSE

SPECIES=MICE SEX=M

PLOT OF MN_FEED*WEEK SYMBOL IS VALUE OF TMT_CODE



NOTE: 6 OBS HAD MISSING VALUES 13 OBS HIDDEN

FIGURE 4

AVERAGE FOOD, WATER, WEIGHT, AND WEIGHT GAIN (GRAMS) BY TREATMENT GROUP
DOXYLAMINE

REPORT: FISH-12
DATE: 09/22/82
TIME: 14:33:37

NTP
EXPERIMENT: 05010 TEST: 02

TEST TYPE: SUBCHRON 90-DAY
PI: DR. C. D. JACKSON, NCTR

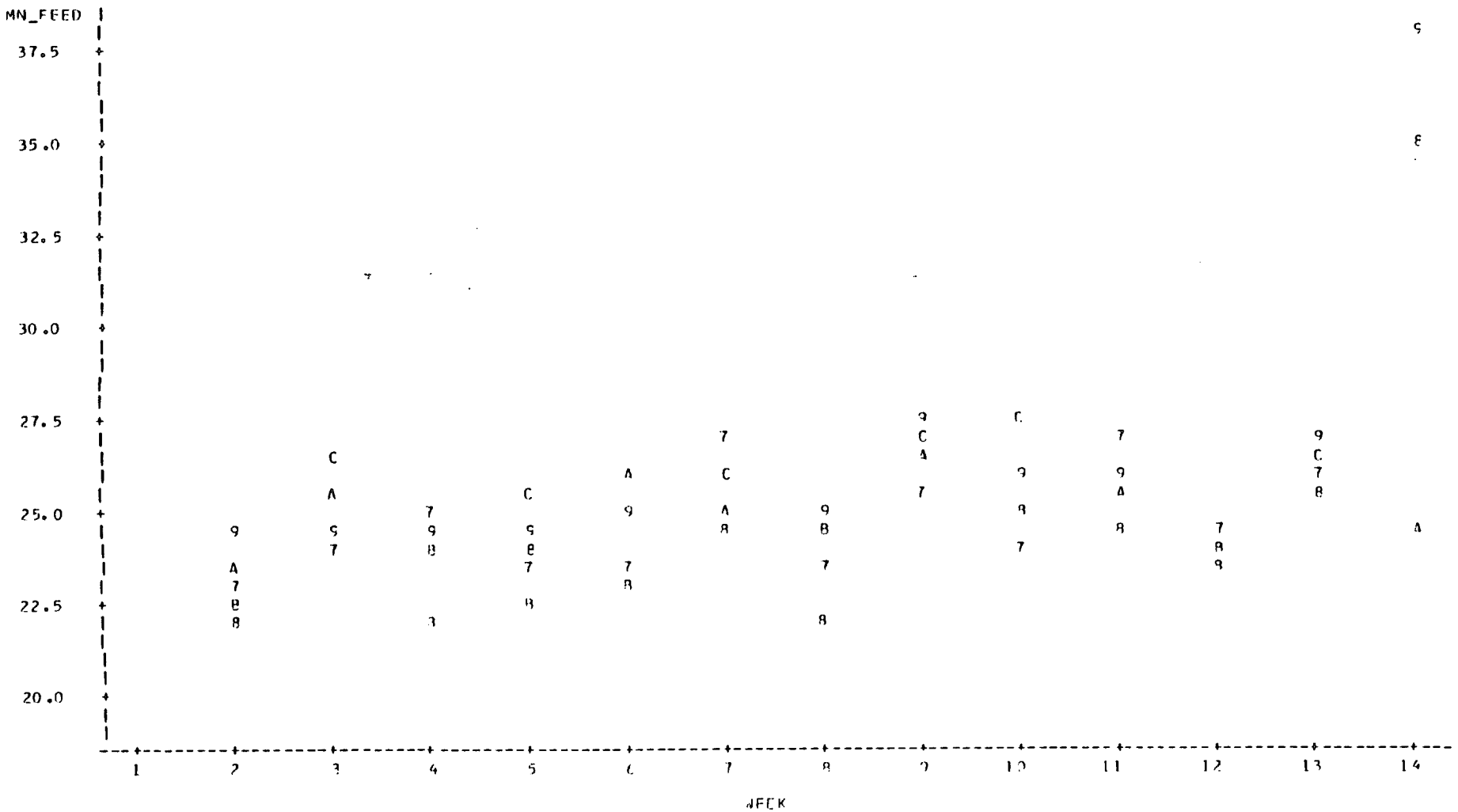
ROUTE: ORAL FOOD

NTP: L0195F
CAS: 00946921C
CONT:

*
PLOT OF AVERAGE FOOD CONSUMPTION (GRAMS/ANIMAL/WEEK) BY DOSE

SPECIES=MICE SEX=F

PLOT OF MN_FEED*WEEK SYMBOL IS VALUE OF TMT_CODE



NOTE: 6 OBS HAD MISSING VALUES 23 OBS HIDDEN

TABLE 4

FINAL BODY WEIGHTS*

Treatment Group	Concentration (ppm)	Least Squares Mean + Standard Error	
		Male	Female
7	Control - 0	31.2 ± 0.3(-)**	24.4 ± 0.31(--)
8	80	33.4 ± 0.31(+7.1)	25.5 ± 0.31(+4.5)
9	162	26.8 ± 0.31(-14.1)	24.9 ± 0.32(+2.1)
10	325	28.4 ± 0.31(-9.0)	23.2 ± 0.32(-4.9)
11	750	29.3 ± 0.31(-6.1)	23.3 ± 0.32(-4.5)
12	1500	28.7 ± 0.13(-8.0)	21.9 ± 0.32(-10.3)

*Final body weights were corrected for differences in initial weights by analysis of covariance. Details of individual data and statistical methods are given in Appendix C.

**Percent difference from controls.

TABLE 5

P VALUES FOR SIGNIFICANT DAY-DOSE EFFECT
BY ONE-WAY ANALYSIS OF VARIANCE
(DOXYLAMINE)

Enzyme	Rats Exp. 376		Mice Exp. 376	
	Male	Female	Male	Female
Aspartate Aminotransferase	NS	.0054	NS	.0001
Alanine Aminotransferase	.0001	.0162	.0001	.0001
Amylase	N/A	N/A	N/A	N/A

NS=not significant at P=.05

G. Organ/Body and Organ/Brain Weight Ratios

Organ weights and their ratios to body weight and brain weight are presented in Appendix C. A number of organ weights and organ/brain weight ratios were found to be significantly different from controls. However, these were scattered and of questionable meaning. There did, however, appear to be an effect of doxylamine on the liver and possibly the kidney. Kidney weights were decreased in males and females when kidney/brain ratios were compared but only in females when kidney/body ratio was used. These effects on kidney occurred only at the highest dose level. A possible increase in thymus was indicated in males but only occurred at 1500 ppm and was not significant at a p value of 0.01.

H. Histopathology Data and Conclusions

Target Organs: In the highest dose group, treatment-related lesions were identified only in the liver. Hence, the liver was examined microscopically in all the animals. Some tissues not specified for examination were inadvertently submitted to the pathologist. These included lung, kidney, spleen, pancreas, preputial gland and mammary gland. These were examined histologically but only those with positive findings were included in the tables in Appendix C.

Lesions and Degree: The liver lesions varied in nature. The lesions consisted of hepatic cell cytomegaly or karyomegaly

which were in varying degree from mild to severe. The lesions were more severe in the higher dose groups and very subtle in the lower dose group. Only the females in the lowest dose group did not have treatment-related lesions. In contrast, the prevalence of a mild degree of hepatic cell necrosis was approximately constant, irrespective of dose. In only three animals was it extensive, one in the highest dose group (1500 ppm) and two in lower dose groups. Hepatic cell cytomegly and/or karyomegaly and hepatic cell necrosis appeared to be dose-related. They were present in approximately equal prevalence and degree in both sexes except that females in the lowest dose group were unaffected. Histopathological findings are presented in Appendix C.

IV. DISCUSSION

Recommendations for the chronic study dosage levels are based on mortality, body weight changes, food consumption, clinical observations, gross observations at necropsy, organ/body weight and organ/brain weight ratios, histopathologic observations, and the results of clinical chemistry. Significant findings in each of these areas are summarized in Table 1 attached to the Abstract of this report.

Significant findings with regard to determining dose levels for the chronic study were primarily reduced weight gain and histopathology, although other data may have supported the significance of these findings. There was little overt toxicity during the 90-day study other than these. Final body weights exhibited a lot of random variation with 162 ppm producing a 14% decrease while 1300 ppm produced only 8% in males. When weight gain was compared, the effects were more exaggerated, but due to the small weight gains in mice, the significance is doubtful. The maximum tolerated dose (MTD) was determined from the final weights after their correction for differences in initial weights of different treatment groups. The scatter of the data made determination of the MTD difficult but values of 160 ppm in males and 650 ppm in females were estimated. However, in choosing a high dose level for the chronic study, the high degree of variability in the data should be kept in mind and the weight curves in Figures 1 and 2 should be considered. The overall shape of the curves indicate less effect of the chemical than a comparison of individual data points suggests.

Histopathology findings suggested that all treated groups, except the very lowest dosed females, were affected by doxylamine. Hepatic cell cytomegaly and/or karyomegaly and hepatic cell necrosis appeared to be dose related and with the exception mentioned, were present in approximately equal prevalence in males and females. Thus, there are pathological manifestations at dose levels below which overt toxic effects, such as weight loss, are observed.

With these considerations, it is recommended that mice in the chronic study be administered 1500 ppm as the high dose and 750 ppm as the low dose.

NATIONAL CENTER FOR TOXICOLOGICAL RESEARCH

FINAL REPORT

EXPERIMENT NO. 376, DOXYLAMINE, 90-DAY STUDY

IN $B6C3F_1$ MICE

APPENDIX A

CHEMISTRY DATA

OPTIONAL FORM NO. 10
JULY 1973 EDITION
GSA FPMR (41 CFR) 101-11.6

UNITED STATES GOVERNMENT

Memorandum

TO : [redacted]
Scientific Intelligence/HFT-30

DATE: July 1, 1981

FROM : Chief, Analytical Methods Branch
Division of Chemistry/HFT-153

SUBJECT: Purity and Characterization Reports on Doxylamine Succinate, Methapyrilene Hydrochloride, and Tripelennamine Hydrochloride; Stability Reports on Doxylamine Hydrochloride and Methapyrilene Hydrochloride.

Please find enclosed the subject reports.

If you have any questions regarding any of the data, please give me a call.

[redacted]

HCTjr/sh
Encls.

5010-110

Buy U.S. Savings Bonds Regularly on the Payroll Savings Plan

CHEMISTRY ANALYSIS - DOXYLAMINE SUCCINATE

I. Purity Analysis of Reference Sample

A. Gas Chromatography

Instrument: Tracor Model 560

Column: 182.9 cm x 2 mm i.d. glass column packed with 5% Dexsil 300 on Chromosorb W 80/100 mesh

Detector: Flame-ionization

Carrier Gas: Helium with a flow rate of 30 ml/min.

Instrument Operating Conditions:

Isothermally - oven temperature 220°C
injector temperature 230°C
detector temperature 250°C

Temperature programmed-injector and detector temperatures same as isothermally
oven temperature programmed from 100-240°C at 10°C/min with a 10 min. hold at final temperature

Results: One major component for Lot Number 001111 after a ten-fold amplification. Isothermally the retention time for the major component was 6.8 minutes. No impurity peaks were detected.

B. High-Pressure Liquid Chromatography

Instrument: Waters Model 6000A solvent delivery system, Tracor Model 970A variable wavelength UV-visible detector, and a Rheodyne septumless injector.

Columns: Waters Bondapak C_{18} (reverse phase) 30 cm x 4 mm i.d., Altex 10 um Lichrosorb guard column 40 mm x 4.6 mm i.d.

Mobile Phase: 80% methanol-20% buffer (0.01M KH_2PO_4, pH 7) flowing at a rate of 1.0 ml/min.

Column Conditions: Ambient temperature

Detector: UV at 254 nm

Results: One major component for Lot Number 001111 with a retention time of 7.4 min. No impurity peaks were detected.

C. Melting Point

Instrument: Thomas Hoover Capillary Melting Point Apparatus

Calibration Standards: Fisher #T-418 TherMetric Standards (benzoic and adipic acid)

Results: Lot Number 001111, 103.5-105°C; Merck Index - 100-104°C

D. Spectral Analysis: IR, UV, MR, NMR

Infrared Analysis: Instrument-Nicolet IR Spectrometer MX-1

Matrix: KBR Pellet

Type of Measurement: % Transmittance

Wavelength Range: 4800-400 cm^{-1}

Resolution: 2 cm^{-1}

The IR spectra was in agreement with that for title compound in the AOAC compilation of IR and UV spectra of some compounds of pharmaceutical interest.

Absorption Maxima: 2650-2800 (broad) cm^{-1}, 1650 cm^{-1}, 1573 cm^{-1}, 1470 cm^{-1}, 1365 cm^{-1}

Ultraviolet Report:

Instrument: Varian 80

Solvent: .01N NaOH; 40 mg/l

$_{max}$: 259 nm

$_{259}$: 4736

Instrument: Cary 17

Solvent: .01N HCl; 10 mg/ml

$_{max}$: 262

$_{262}$: 10689

The UV spectra was in agreement with that published for title compound in the AOAC compilation of IR and UV spectra of some compounds of pharmaceutical interest.

Mass Spectral Analysis

Instrument: Finnigan 4023 Quadrupole

Mode: Electron Impact-70eV

Inlet: Direct Probe

The succinate thermally decomposed to the free amine upon heating in vacuo, therefore, it was converted to the free base by treatment with NH_4OH followed by extraction with CH_2Cl_2. The mass spectrum was consistent with the library spectra of N,N-Dimethyl-2-/1-phenyl-1-(2-pyridinyl)ethoxylethaneamine. The base peak in the spectrum, m/z 58, represents homolytic cleavage of the ethane 1,2 bond. The molecular ion was observed as the protonated species m/z 271. An ion produced by rupture of the O-CH_2 bond with a double hydrogen

rearrangement to yield m/z 200 was observed. Subsequent loss of H_2O and CH_3 from m/z 200 and 182 were observed. The ion m/z 71 resulted from cleavage about the O-CH_2 bond with charge retention on the amine moiety.

Structure Analysis by NMR Spectroscopy

Instrument: Bruker WH270

Configuration: ^{1}H, ^{13}C

The ^{13}C chemical shifts of the sample were measured, and compared with the ^{13}C NMR data of the model compounds 2-pyridinyl carbanol, 1-phenylethanol and 2-(N,N-dimethylamino)-ethanol as well as succinate ("^{13}C NMR Satler Index"). The chemical shift data for corresponding nuclei was in good agreement. The ^{1}H NMR chemical shift and associated coupling patterns, determined from homonuclear decoupling experiments and spectral integrations, were consistent with the doxylamine succinate structure. No impurities were detected.

II. Purity Analysis of Bulk Sample

A. Gas Chromatography

Instrument: Tracor Model 560

Column: 182.9 cm x 2 mm i.d. glass column packed with 5% Dexsil 300 on Chromosorb W 80/100 mesh.

Detector: Flame-ionization

Carrier Gas: Helium with a flow rate of 30 ml/min.

Instrument Operating Conditions:

Isothermally-oven temperature 220°C
-injector temperature 230°C
-detector temperature 250°C

Temperature programmed -injector and detector temperature same as isothermally
-oven temperature programmed from 100-240°C at 10°C/min with a 10 min. hold at final temperature.

Results: One major component for Lot Number L0536-1 after a ten-fold amplification. Isothermally the retention time for the major component was 6.8 minutes. No impurity peaks were detected.

B. High Pressure Liquid Chromatography

Instrument: Waters Model 6000A solvent delivery system, Tracor Model 970A variable wavelength UV-visible detector, and a Rheodyne septumless injector

Columns: Waters Bondapack C_{18} (reverse phase) 30 cm x 4 mm i.d., Altex 10 um Lichrosorb guard column 40 mm x 4.6 mm i.d.

Mobile Phase: 80% methanol-20% buffer (0.01 M KH_2PO_4, pH 7) flowing at a rate of 1.0 ml/min.

Column Conditions: Ambient temperature

Detector: UV at 254 nm

Results: One major component for Lot Number LO 536-1 with a retention time of 7.4 min. No impurity peaks were detected.

C. Melting Point

Instrument: Thomas Hoover Capillary Melting Point Appartus

Calibration Standards: Fischer #T-418 TherMetric Standards (benzoic and adipic acid)

Results: Lot Number LO5361, 104-105.5°C; Merck Index 100-104°C

D. Vacuum Volatiles Determination: Vacuum volatiles were determined on Lot Number 536-1. Five grams of the chemical were analytically weighed into an aluminum foil pan and placed in an oven at 60°C under 18 inches of vacuum for 16 hours. The sample was removed from the oven and cooled in a desiccator. The sample was then weighed to determine the weight loss. Duplicate determinations were performed.

E. Spectral Analysis: IR, UV, MS, NMR

Infrared Analysis

Instrument: Nicolet IR Spectrometer MX-1

Matrix: KBR Pellet

Type of Measurement: % Transmittance

Wavelength Range: 4800-400 cm^{-1}

Resolution: 2 cm^{-1}

The IR spectra was in agreement wit that for the test compound in the AOAC compilation of IR and UV spectra of some compounds of pharmaceutical interest, revised edition.

Absorption Maxima: 1745 cm^{-1}, 1650 cm^{-1}, 1600 cm^{-1}, 1477 cm^{-1}, 1325 cm^{-1}, 2778-2439 (broad cm^{-1}).

Ultraviolet Analysis

Instrument: Varian 80

Solvent: .01N NaOH; 40 mg/l

$_{max}$: 259 nm

$_{259}$: 4467

Instrument: Cary 17

Solvent: .01N HCl; 10 mg/ml

$_{max}$: 262

$_{262}$: 10431

The UV spectra was in agreement with that published for title compound in the AOAC compilation of IR and UV spectra of some compounds of pharmaceutical interest.

Mass Spectral Analysis

Instrument: Finnigan 4023 Quadrupole

Mode: Electron Impact-70eV

Inlet: Direct Probe

The succinate thermally decomposed to the free amine upon heating in vacuo, therefore, it wa converted to the free base by treatment with NH_4OH followed by extraction with CH_2Cl_2. The mass spectrum was consistent with the library spectra of N,N-Dimethyl-2-/1-phenyl-1-(2-pyridinyl)ethoxylethaneamine. The base peak in the spectrum, m/z 58, represents homolytic cleavage of the ethane 1,2 bond. The molecular ion was observed as the protonated species m/z 271. An ion produced by rupture of the $O-CH_2$ bond with a double hydrogen rearrangement to yield m/z 200 was observed. Subsequent loss of H_2O and CH_3 from m/z 200 and 182 were observed. The ion m/z 71 resulted from cleavage about the $O-CH_2$ bond with charge retention on the amine moiety.

Structure Analysis by NMR Spectroscopy

Instrument: Bruker WH270

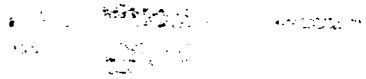
Configuration: ^{1}H, ^{13}C

The ^{13}C chemical shifts of the sample were measured, and compared with the ^{13}C NMR data of the model compounds 2-pyridinyl carbanol, 1-phenylethanol and 2-(N,N-dimethylamino)-ethanol as well as succinate ("^{13}C NMR Satler Index"). The chemical

shift data for corresponding nuclei was in good agreement. The ^{1}H NMR chemical shift and associated coupling patterns, determined from homonuclear decoupling experiments and spectral integrations, were consistent with the doxylamine succinate structure. No impurities were detected.

III. CONCLUSIONS

Both high-pressure liquid chromatography and gas chromatography using flame-ionization detection indicated one major component for both the reference chemical and the bulk material. The melting points were consistent with the literature value. The nuclear magnetic resonance, infrered, ultraviolet and mass spectra were consistent with the structure of doxylamine succinate. It was concluded that both the reference chemical and the bulk mamerial (lots 001111 and LO 536-1, respectively) were doxylamine succinate. Both lots can be considered essentially pure based on the analyses performed.



STABILITY STUDIES

Since the test compound was to be administered as a mixture in animal feed, the stability of doxylamine succinate was determined under conditions simulating the conditions of feed mixing, storage, and administration. Feed was dosed by the SOP (Standard Operating Procedure) with doxylamine succinate (Lot Number LO 536-1) at levels of 0, 100, and 2000 ppm on the basis of the free amine. Both the short- and long-term stability was determined.

The feed used in the short-term study (simulated animal test conditions) was placed in crystallizing dishes and allowed to stand in the open vessel in a fume hood under incandescent lighting at ambient temperature. The feed used in the long-term study (simulated feed storage conditions) was sealed in amber bottles and stored in a light free cabinet at ambient temperatures. Duplicate samples from each level for both the short- and long-term study were assayed at the intervals indicated below:

Assay Method: High Pressure Liquid Chromatography

Instrument: Waters Model 6000A pump, Tracor Model 970A variable wavelength UV-visable detector, Rheodyne Model 7120 septumless injector.

Column: Waters uBondapak C_{18} (reverse phase) 30 cm x 4 mm i.d., Altex 10 um lichrosorb guard column 40 mm x 4.6 mm i.d.

Mobile Phase: 90% methanol-10% buffer (0.01M KH_2PO_4, pH 7) flowing at a rate of 1 ml/min.

Column Conditions: Ambient temperature.

Detector: UV at 254 nm

Results:

	Short-Term Study	
	Dose Levels (ppm)*	
Days	100	2000
0	100 ± 2	2000 ± 50
1	103 ± 0	1990 ± 40
2	102 ± 1	2020 ± 20
5	102 ± 1	2020 ± 0
8	104 ± 4	2000 ± 30
36	99 ± 3	**

	Long-Term Study	
	Dose Levels (ppm)*	
Weeks	100	2000
0	100 ± 2	2000 ± 50
1	101 ± 2	1990 ± 10
2	101 ± 0	1960 ± 40
4	**	**
8	98 ± 2	1990 ± 20
16	102 ± 1	2000 ± 20

*Samples are corrected for background and recovery.

**Not determined.

Although an analysis was not performed on the long-term stability study sample (2000 ppm level) after 36 days, it was deduced that the high level is as stable as the low level sample for the 36-day period. After completing the stability tests for doxylamine succinate in animal feed, it was concluded that the chemical is stable in this substrate for a sufficient period of time to allow administration of the chemical to test animals via feed.

DOSE ANALYSIS

A twenty g sample of feed was mixed with 20 ml of 1N HCl and 80 ml of methanol in a 250 ml Erlenmeyer flask fitted with a Teflon-lined screw cap. The sample was mechanically extracted for one hour on a reciprocating shaker at a rate of 2000 excrusions/min. and allowed to stand approximately 10 min. The supernatant was decanted into a 50 ml cultuer tube and centrifuged at 1000 rpm. A 5 ml aliquot of the supernatant (equal to 1 g feed) was transferred to a 30 ml culture tube containing 10 ml of 1 N HCl and 10 ml of dichloromethane. The contents were shaken vigorously and centrifuged for 2 min. at 1000 rpm. The dichloromethane was withdrawn with a syringe filtered with a canula and discarded. The aqueous layer was extracted with two additional 10 ml portions of dichloromethane which were also discarded. (Care was taken not to remove any of the aqueous phase.) Two ml of 1 M dibasic potassium phosphate (pH 9.4) and 1.1 ml of 10 N NaOH were added and the aqueous layer was extracted three times with 10 ml portions of dichloromethane which were successively percolated through a plug of anhydrous sodium sulfate(ca. 18 mm diameter x 30 mm thick) into a 50 ml round-bottom flask. The combined extracts were evaporated to dryness at ambient tmeperature with a rotary evaporator and water pump vacuum. The residue was reserved for cleanup on a silica gel column.

The silica gel columns (12 mm i.d., Konter Glass Co., Vineland, NJ) were prepared just prior to using by successively adding a plug of glass wool, 2 g of amhydrous sodium sulfate, 2 g of silica gel (5% water) and 2 g of anhydrous sudium sulfate. The columns were washed with four 5 ml portions of dichloromethane (0.01 M triethylamine, TEA) whcih were discarded. The residue from the feed extraction was dissolved in 2 ml dichloromethane (0.01 M TEA) transferred to a silica gel (5% water) column and four additional 2 ml portions of dichlorometahen (0.01M TEA) were used to wash the flask and transfer the residue to the column quantitatively. The column eluate was discarded and the column was washed with two 5 ml portions of 5% methanol in dichloromethane (0.01 M TEA). The doxylamine free base was eluted with 15 ml of 5% methanol in dichloromethane (0.01 M TEA) and collected in a 50 ml round-bottom flask. The sample was evaporated to dryness at ambient temperature with a rotary evaporator and water pump vacuum. The residue was dissolved in 1 ml or more of the appropriate solvent for analysis by HPLC or N/P-GC.

The HPLC system consisted of a Waters Associates, Inc., Model 6000A solvent delivery system, a Rheodyne, Inc. Model 7120 systemless injector, an Altex 5 uODS ultrasphere column, 25 cm x 4.6 mm i.d., and a Tracor, Inc. Model 970A variable wavelength UV-visible absorbance detector set at 254 nm. The mobile phase was 90% methanol-10% aqueous monobasic potassium phosphate (0.01M, pH 7 adjusted with 10 N NaOH) which flowed at a rate of 1 ml/min. at a pressure of 800 psi. The retention time for doxylamine was 5.0 min. Doxylamine residues in feed at levels of 10, 100, and 11000 ppm, each containing 1g equivalent of animal feed from the silica gel column cleanup were dissolved in 1 ml or more of methanol. Injections of 20 ul of the methanol solutions were made and compared to similar injections of standard solutions of doxylamine succinate.

Results: During the course of the study four standard solutions were made up for the preparation of dosed feed. These were analyzed by the Chemistry Division to confirm the dose levels. The results were as follows:

Sample Identification No.	Stock Solution Batch No.	Target Concentration	Assay Results
52-231-1287	81-06-27-004	10%	10.20%
52-231-1288	81-97-28-005	10%	9.73%
52-155-184	81-08-25-006	10%	9.52%
52-231-1574	81-08-25-006*	10%	10.20%
52-155-192	81-09-17-007	10%	9.88%

*Batch 81-08-25-006 was reassayed when the first assay indicated a low concentration.

The results confirmed the concentrations of the stock solutions and they were each used to prepare dosed feed. A number of batches of dosed feed were analyzed to confirm dose levels and to serve as a quality control on diet preparations. The batches of diet analyzed in this study and the results are given on page 28.

Minor deviations from the ± 10% variation standard for dose-levels were noted but were considered acceptable due to the preliminary nature of the study.

DOSED FEED ANALYSIS

Sample Identification Number	Dosed Feed Batch Number	Target Concentration[1]	Assay Results
52-231-1070	81-06-30-011	80	92.6
52-231-1071	81-06-30-012	162	156
52-231-1072	81-06-30-013	162	144
52-231-1073	81-06-30-014	325	310
52-231-1076	81-06-01-017	750	676
52-231-1076	81-07-01-017	750	676
52-231-1079	81-07-01-020	1500	1380
52-231-1296	81-07-30-026	162	143
52-231-1475	81-08-27-035	162	152
52-231-1501	81-08-31-040	1500	1560
52-231-1503	81-09-92-041	162	163
52-231-1607	81-09-18-043	80	83
52-231-1608	81-09-18-043	80	83
52-231-1609	81-09-18-044	162	170
52-231-1610	81-09-18-043	162	147

[1]Concentrations are expressed as ppm and refer to the free amine, not the succinate derivative.

NATIONAL CENTER FOR TOXICOLOGICAL RESEARCH

FINAL REPORT

EXPERIMENT NO. 376, DOXYLAMINE, 90-DAY STUDY

IN $B6C3F_1$ MICE

APPENDIX B

ANIMAL DATA

NATIONAL CENTER FOR TOXICOLOGICAL RESEARCH
CHEMISTRY QUERY REPORT

Jells

RT NO. CH-026

Waste water -
Potable water -

DATE 01/22/81
TIME 10:58:07
PAGE 1

EQ 71126 AND SEQ EQ 00375

CH NUMBER	NCTR SID (CONTR ID)	EXP	LOGIN	COMPL	TYPE	SUBTYPE
1	71126000375	022	80317	81022	WATER/SOLNS	HEATED, FILTERED

#REP 02
COLL 801110

	DOSE	SPECIFICATIONS MIN	SPECIFICATIONS MAX	SPECIFICATIONS MDL	OBSERVED CONC
07 ARSENIC, PPB				20.	<MDL
10 CADMIUM, PPB				5.	<MDL
14 DDT, TOTAL, PPB				50.	<MDL
29 LEAD, PPM				0.02	<MDL
33 MERCURY, PPM				0.02	<MDL
36 OP PESTICIDES, PPB				50.	<MDL
37 PCB, PPB				50.	<MDL
44 SELENIUM, PPM				0.02	<MDL
67 OC PESTICIDES, PPM				0.05	<MDL
68 IRON, PPM				0.02	0.04
69 NICKEL, PPM				0.02	<MDL
70 BERYLLIUM, PPM				0.02	<MDL
71 CHROMIUM, PPM				0.02	<MDL
72 COPPER, PPM				0.02	0.046
73 CHLOROFORM, PPM				0.001	<MDL

50 POUNDS NET WEIGHT

5010

AUTOCLAVABLE RODENT LABORATORY CHOW®
#5010

Animal Diet

GUARANTEED ANALYSIS

Crude protein not less than	23.0%
Crude fat not less than	4.5%
Crude fiber not more than	6.0%
Ash not more than	8.0%
Added minerals not more than	3.0%

INGREDIENTS

Ground extruded yellow corn, soybean meal, wheat middlings, fish meal, wheat germ meal, ground oat groats, brewers' dried yeast, soybean oil, dehydrated alfalfa meal, dried beet pulp, ground wheat, calcium carbonate, dicalcium phosphate, salt, animal liver meal, vitamin B-12 supplement, biotin, methionine hydroxy analogue calcium, calcium pantothenate, choline chloride, folic acid, riboflavin supplement, D activated animal sterol (source of vitamin D-3), vitamin A supplement, vitamin E supplement, thiamin, niacin, pyridoxine hydrochloride, menadione sodium bisulfite (source of vitamin K activity), silicon dioxide, calcium iodate, manganous oxide, copper sulfate, zinc oxide.

T-5010

(Continued - See Reverse Side)

5010

Ralston Purina Co.,
Gen. Offices, St. Louis, MO 63188

MEAL

Autoclavable Rodent Laboratory Chow 5010

FEEDING DIRECTIONS

Feed ad libitum to rodents. Plenty of fresh, clean water should be available to the animals at all times.

Rats - Adult rats will eat 12 to 15 grams of Rodent Laboratory Chow® animal diet per day. Feeders in rat cages should be designed to hold two to three days' supply of feed at one time.

Mice - Adult mice will eat 4 to 5 grams of diet daily. Some of the larger strains may eat as much as eight grams per day per animal. Feed should be available on a free choice basis in wire feeders above the floor of the cage.

Hamsters - Adults will eat 10 - 14 grams per day.

CAUTION

Feed is perishable. Store in a dry, well ventilated area, free of pests and insects. Do not use moldy or insect-infested feed.

IMPORTANT

The feeding program is only as effective as the management practices followed.

AUTOCLAVING SUGGESTIONS

During the autoclaving process the pellets can be placed on trays, in small bags or in larger bags as long as the pellets are stacked no more than 3 inches high.

When steam autoclaved, the pellets swell and exert force on adjacent pellets. If confined by a bag or container, the pressure causes sticking as greater polymerization of fibrous materials occur under such conditions.

ASSAY BEFORE AND AFTER AUTOCLAVING

Conditions of sterilization must be determined for each autoclaving unit. It is best to assay the diet before and after sterilization to determine nutrient losses. Microbiological studies should be done also to insure the degree of sterilization desires.

NOT FOR SALE

NATIONAL CENTER FOR TOXICOLOGICAL RESEARCH
CHEMISTRY QUERY REPORT

REPORT NO. CH-026

Feed Lot

DATE 04/17/81
TIME 10:04:02
PAGE 1

ARE EQ 74109 AND SEQ GE 00003 AND SEQ LE 00006

BATCH NUMBER	NCTR SID (CONTR ID)	EXP	LOGIN	COMPL	TYPE	SUBTYPE
1						
2000000	81011512	74109100006	145	81022	81107 FEED	SHIPMENT LOT

* MTYP 02
COLL 810122

	DOSE	-----SPECIFICATIONS----- MIN	MAX	MDL	OBSERVED CONC
05 AFLATOXIN, PPB			5.		<MDL
07 ARSENIC, PPB			1000.	20.	560
10 CADMIUM, PPB			250.	5.	<MDL
14 DDT, TOTAL, PPB			100.	5.	<MDL
16 DIELDRIN, PPB			20.	5.	<MDL
29 LEAD, PPM			1.5		0.56
31 LINDANE, PPB			100.	1.	<MDL
32 MALATHION, PPB			5000.	50.	70
33 MERCURY, PPM			0.2	0.02	<MDL
43 PROTEIN, TOTAL, %		21.	28.		24.0
44 SELENIUM, PPM		0.05	0.65		0.34
58 VITAMIN A, IU/G		15.	75.		33.0
59 VITAMIN B1, MG/G		0.075	0.125		0.079
74 TOTAL FAT, %		4.3	6.7		5.0
127 NITROSAMINES, PPB				0.10	13
128 HEPTACHLOR EPOXIDE,PPB				0.05	<MDL

2						
2000001	81011425	74109100004	145	81022	81107 FEED	SHIPMENT LOT

MTYP 01
COLL 810122

	DOSE	-----SPECIFICATIONS----- MIN	MAX	MDL	OBSERVED CONC
05 AFLATOXIN, PPB			5.		<MDL
07 ARSENIC, PPB			1000.	20.	500
10 CADMIUM, PPB			250.	5.	<MDL
14 DDT, TOTAL, PPB			100.	5.	<MDL
16 DIELDRIN, PPB			20.	5.	<MDL
29 LEAD, PPM			1.5		0.54
31 LINDANE, PPB			100.	1.	<MDL
32 MALATHION, PPB			5000.	50.	70
33 MERCURY, PPM			0.2	0.02	<MDL
37 PCB, PPB			50.	10.	<MDL
43 PROTEIN, TOTAL, %		21.	28.		23.4
44 SELENIUM, PPM		0.05	0.65		0.36
58 VITAMIN A, IU/G		15.	75.		26.0
59 VITAMIN B1, MG/G		0.075	0.125		0.075
74 TOTAL FAT, %		4.3	6.7		5.0
127 NITROSAMINES, PPB				0.10	11
128 HEPTACHLOR EPOXIDE,PPB				0.05	<MDL

NATIONAL CENTER FOR TOXICOLOGICAL RESEARCH
CHEMISTRY QUERY REPORT

EPORT NO. CH-026

DATE 06/17/81
TIME 09:27:30
PAGE 3

TYP EQ 02 AND AN EQ 74 AND CMP EQ 81168

BATCH NUMBER	NCTR SID (CONTR ID)	EXP	LOGIN	COMPL	TYPE	SUBTYPE
5						
2000000081031716	74109100025	145	81083	81168	FEED	SHIPMENT LOT

5010-M

MTYP 02
COLL 810324

	DOSE	SPECIFICATIONS MIN	SPECIFICATIONS MAX	MDL	OBSERVED CONC
05 AFLATOXIN, PPB			5.		<MDL
07 ARSENIC, PPB			1000.	20.	270
10 CADMIUM, PPB			250.	5.	<100
14 DDT, TOTAL, PPB			100.	5.	<10
16 DIELDRIN, PPB			20.	5.	<10
29 LEAD, PPM			1.5		0.62
31 LINDANE, PPB			100.	1.	<10
32 MALATHION, PPB			5000.	50.	90
33 MERCURY, PPM			0.2	0.02	<0.05
37 PCB, PPB			50.	10.	<200
43 PROTEIN, TOTAL, %		21.	28.		23.2
44 SELENIUM, PPM		0.05	0.65		0.42
58 VITAMIN A, IU/G		15.	75.		27.0
59 VITAMIN B1, MG/G		0.075	0.125		0.083
127 NITROSAMINES, PPB				0.10	1.2
128 HEPTACHLOR EPOXIDE,PPB				0.05	<10
74 TOTAL FAT, %		4.3	6.7		5.5

6						
2000000	81031717	74109100026	145	81083	81168	FEED SHIPMENT LOT

5010-M

MTYP 02
COLL 810324

	DOSE	SPECIFICATIONS MIN	SPECIFICATIONS MAX	MDL	OBSERVED CONC
05 AFLATOXIN, PPB			5.		<MDL
07 ARSENIC, PPB			1000.	20.	150
10 CADMIUM, PPB			250.	5.	<100
14 DDT, TOTAL, PPB			100.	5.	<10
16 DIELDRIN, PPB			20.	5.	<10
29 LEAD, PPM			1.5		0.52
31 LINDANE, PPB			100.	1.	<10
32 MALATHION, PPB			5000.	50.	110
33 MERCURY, PPM			0.2	0.02	<0.05
43 PROTEIN, TOTAL, %		21.	28.		23.2
44 SELENIUM, PPM		0.05	0.65		0.39
58 VITAMIN A, IU/G		15.	75.		27.0
59 VITAMIN B1, MG/G		0.075	0.125		0.081
127 NITROSAMINES, PPB				0.10	1.7
128 HEPTACHLOR EPOXIDE,PPB				0.05	<10
37 PCB, PPB			50.	10.	<200
74 TOTAL FAT, %		4.3	6.7		5.3

@ 74109 AND SEQ GE 00031 AND SEQ LE 00040

BATCH NUMBER	NCTR SID (CONTR ID)	EXP	LOGIN	COMPL	TYPE	SUBTYPE
3						
000000081052013	74109100034	145	81156	81202	FEED	SHIPMENT LOT

MTYP 02
COLL 810603

	DOSE	SPECIFICATIONS MIN	SPECIFICATIONS MAX	MDL	OBSERVED CONC
05 AFLATOXIN, PPB			5.		<5
07 ARSENIC, PPB			1000.	20.	420
10 CADMIUM, PPB			250.	5.	<100
14 DDT, TOTAL, PPB			100.	5.	<10
16 DIELDRIN, PPB			20.	5.	<10
29 LEAD, PPM			1.5		0.28
31 LINDANE, PPB			100.	1.	<10
32 MALATHION, PPB			5000.	50.	<50
33 MERCURY, PPM			0.2	0.02	<0.05
37 PCB, PPB			50.	10.	<50
43 PROTEIN, TOTAL, %		21.	28.		23.5
44 SELENIUM, PPM		0.05	0.65		0.34
58 VITAMIN A, IU/G		15.	75.		29.00
59 VITAMIN B1, MG/G		0.075	0.125		0.11
74 TOTAL FAT, %		4.3	6.7		4.8
127 NITROSAMINES, PPB				0.10	1.3
128 HEPTACHLOR EPOXIDE,PPB				0.05	<10

4						
200000[81052018]	74109100033	145	81156	81202	FEED	SHIPMENT LOT

MTYP 02
COLL 810603

	DOSE	SPECIFICATIONS MIN	SPECIFICATIONS MAX	MDL	OBSERVED CONC
05 AFLATOXIN, PPB			5.		<5
07 ARSENIC, PPB			1000.	20.	430
10 CADMIUM, PPB			250.	5.	<100
14 DDT, TOTAL, PPB			100.	5.	<10
16 DIELDRIN, PPB			20.	5.	<10
29 LEAD, PPM			1.5		0.42
31 LINDANE, PPB			100.	1.	<10
32 MALATHION, PPB			5000.	50.	<50
33 MERCURY, PPM			0.2	0.02	<0.05
37 PCB, PPB			50.	10.	<50
43 PROTEIN, TOTAL, %		21.	28.		23.5
44 SELENIUM, PPM		0.05	0.65		0.22
58 VITAMIN A, IU/G		15.	75.		29.00
59 VITAMIN B1, MG/G		0.075	0.125		0.11
74 TOTAL FAT, %		4.3	6.7		4.8
127 NITROSAMINES, PPB				0.10	1.3
128 HEPTACHLOR EPOXIDE,PPB				0.05	<10

RE EQ 74109 AND SEQ GE 00043 AND SEQ LE 00056

BATCH NUMBER	NCTR (CONTR ID)	SID EXP	LOGIN	COMPL	TYPE	SUBTYPE
5						
2000000 81061626	74109100046	145	81177	81231	FEED	SHIPMENT LOT

MTYP 02
COLL 810624

5010M

	DOSE	SPECIFICATIONS MIN	SPECIFICATIONS MAX	MDL	OBSERVED CONC
05 AFLATOXIN, PPB			5.		<5
07 ARSENIC, PPB			1000.	20.	390
10 CADMIUM, PPB			250.	5.	<100
14 DDT, TOTAL, PPB			100.	5.	10
16 DIELDRIN, PPB			20.	5.	<10
29 LEAD, PPM			1.5		0.80
31 LINDANE, PPB			100.	1.	<10
32 MALATHION, PPB			5000.	50.	90
33 MERCURY, PPM			0.2	0.02	<0.05
37 PCB, PPB			50.	10.	<50
43 PROTEIN, TOTAL, %		21.	28.		24.1
44 SELENIUM, PPM		0.05	0.65		0.32
58 VITAMIN A, IU/G		15.	75.		28.00
59 VITAMIN B1, MG/G		0.075	0.125		0.11
74 TOTAL FAT, %		4.3	6.7		5.2
127 NITROSAMINES, PPB				0.10	2.7
128 HEPTACHLOR EPOXIDE,PPB				0.05	<10

BATCH NUMBER	NCTR (CONTR ID)	SID EXP	LOGIN	COMPL	TYPE	SUBTYPE
6						
2000000 81061627	74109100047	145	81177	81231	FEED	SHIPMENT LOT

MTYP 02
COLL 810624

5010M

	DOSE	SPECIFICATIONS MIN	SPECIFICATIONS MAX	MDL	OBSERVED CONC
05 AFLATOXIN, PPB			5.		<5
07 ARSENIC, PPB			1000.	20.	400
10 CADMIUM, PPB			250.	5.	<100
14 DDT, TOTAL, PPB			100.	5.	10
16 DIELDRIN, PPB			20.	5.	<10
?•?•?• 29 LEAD, PPM			1.5		1.94
31 LINDANE, PPB			100.	1.	<10
32 MALATHION, PPB			5000.	50.	100
33 MERCURY, PPM			0.2	0.02	<0.05
37 PCB, PPB			50.	10.	<50
43 PROTEIN, TOTAL, %		21.	28.		24.5
44 SELENIUM, PPM		0.05	0.65		0.26
58 VITAMIN A, IU/G		15.	75.		29.00
59 VITAMIN B1, MG/G		0.075	0.125		0.11
74 TOTAL FAT, %		4.3	6.7		4.9
127 NITROSAMINES, PPB				0.10	3.1
128 HEPTACHLOR EPOXIDE,PPB				0.05	<10

See Correction Sheet Dated 11/16/81

?•?•?• OUTSIDE SPECIFICATIONS

MEMORANDUM [redacted] DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE
PUBLIC HEALTH SERVICE
[redacted]OD AND DRUG ADMINISTRATION

TO : Principal Investigator, E-376 DATE: 9/4/81

FROM : Chief, Diet Preparation Branch (HFT-213 [redacted]

SUBJECT: Delivery of Microbiologically uncertified feed to animals.

Present circumstances indicate that it is necessary for Diet Prep to deliver uncertified feed to your animals, or place these animals on control feed until certified feed can be prepared.

Please note the following, specify your wishes:

Reason: Micro assay showed 10⁸ bacteria (8 over std) PI was called, no reply - contractor was waiting to pack out or re-mix. I chose to pack out and ship.

Actions: (1) Use uncertifi[redacted] is available.

Signature Date Chief, Diet Prep.

(2) Use control feed until certified feed is available. _____

Signature Date

Eight Bacteria, non pathogenic, could not pose any sort of problem for this lot of these animals. I made the decision to use the feed rather than discard and remix. Save chemical, time and money which we are [redacted] short of at Presa

MEMORANDUM

DEPARTMENT OF HEALTH AND HUMAN SERVIC
PUBLIC HEALTH SERVICE
FOOD AND DRUG ADMINISTRATION

TO: [redacted]
HFT-30

DATE: 5-3-82

FROM: Director, Division of Microbiological Services
HFT-250

SUBJECT: Microbiological Evaluation of Fourteen NTP Studies and Breeder Animals

The SPF breeder mice and rats used for all NTP studies have been microbiologically evaluated since January 1981. In addition, direct environmental samples which include feed, cage water, bedding and waste and room air samples have been microbiologically evaluated since January 1981.

As of April 1, 1982, 21 female mice from strain 1B (C57BL/6N) and 21 male mice from strain 04 (C3H/HeN MTV$^-$) were evaluated for primary potential pathogens (see Table I). These two strains were the direct parents of strain 1H (B6C3F1) that were used for NTP experiments. Two 1B strain parents were positive for Reo-3 virus with low titers of 1:20 and 1:40. One strain 04 parent was positive for Encephalomyelitis virus (GDVII) with a low titer of 1:40. No other potential microbial pathogen were identified or isolated.

As of April 1, 1982, two hundred fifty-seven strain Fisher 344(1A) rats, which were obtained from the same cages as the animals which were used for the NTP studies, were microbiologically evaluated. Only one rat was positive for Reo-3 virus with a low titer of 1:20. No other potential microbial pathogens were identified or isolated.

Environmental samples from all breeder rooms were taken on a weekly basis and consisted of feed, cage water, bedding and waste, room air samples and room and equipment swab samples. All samples were negative for potential pathogenic organisms or did not exceed microbial standards for non-pathogenic organisms.

As of April 1, 1982, environmental samples have been obtained and assayed from Experiments 357, 358, 359, 369, 373, 375, 376, 379, 382, 383, 396, 397, 399 and 400. Composite bedding and waste samples, room air samples and cage water samples (one rack) were sampled from each room on a monthly basis. Approximately two feed samples per compound/per experiment were sampled on a weekly basis. Room swab samples were evaluated prior to initiation of experiments and on a quarterly basis if the experiment lasted that long. Samples from the following experiments were considered unacceptable and final disposition is indicated.

Experiment No. 359 - One feed sample contained excessive mold concentration. The feed batch was discarded.

POTENTIAL PATHOGENIC MICROORGANISMS OF PRIMARY CONCERN IN SURVEILLANCE ANIMAL AND NON-ANIMAL SAMPLES

BACTERIA

Salmonella spp.

S. pneumoniae

S. moniliformia

S. pyogenes (Gp. A)

B. piliformis

C. kutscheri

C. freundii

B. bronchiseptica

P. aeruginosa

Pasteurella spp.

K. pneumoniae

FUNGI

B. dermatitidis

H. capaulatum

C. neoformis

MYCOPLASMA

M. pulmonis

M. arthritidis

M. neurolyticum

VIRUSES

Reo-3

LCM

Sendai

PVM

MHV

Polyoma

Ectromelia

LDV

GDVII

PARASITES

All Ectoparasites

Endoparasites:

Syphacia obvelata

Aspicul. tetraptera

Hexamita muris

Hymenolepsis sp.

Protozoa:

Encephalitozoon sp.

Toxoplasma gondii

Rickettsia:

Eperythrozoon sp.

Haemobartonella sp.

* Other microbial agents were included as deemed necessary dependent upon the test chemical and stress situation.

Experiment No. 369 - One feed sample contained excessive bacterial concentration. The feed batch was discarded.

Experiment No. 376 - Four feed samples contained excessive bacterial concentration. One feed sample contained excessive bacterial and mold concentration. All five feed batches were discarded. One hundred thirty-one cage water samples were cultured for Pseudomonas (Ps.) aeruginosa only. One cage was positive. It was recommended that the animals in that cage be destroyed.

Experiment No. 382 - Ninety-six cage water samples were cultured for Ps. aeruginosa only. Six cages were positive. It was recommended that the animals in those cages be destroyed. One environmental bedding and waste sample from one of the above positive water samples was also positive for Ps. aeruginosa.

Environmental samples taken from all other experiments were considered acceptable. No animals were removed from any of the experiments for microbiological evaluation.

[redacted]

cc: [redacted]

MEMORANDUM

DEPARTMENT OF HEALTH AND HUMAN SERVICES
PUBLIC HEALTH SERVICE
FOOD AND DRUG ADMINISTRATION

DATE: FEB 04 1982

TO: [redacted]

FROM: Director, Division of Microbiology
HFT-160

SUBJECT: Microbiological Survey - Weekly Report

EXPERIMENT NO. 376

Deoxylamine Sub-Chronic--Mice & Rats

FOR THE WEEK STARTING: JAN 24 1982

The attached report contains laboratory determinations made on animal and non-animal specimens and biological indicators from the experiment listed above. Pending data (incomplete laboratory results), when available, will be included in subsequent weekly reports. Any questions, comments, etc., should be directed to this office.

[redacted]

Bldg. 71-Room 151

cc: [redacted]

02/03/82
REPORT NO. CS003

NATIONAL CENTER FOR TOXICOLOGICAL RESEARCH
MICROBIOLOGICAL SERVICES BRANCH

NON-ANIMAL REPORT

EXPERIMENT NO. 376

W K STARTING 01/24/82

SIC	DATE RECEIVED	TYPE/SUBTYPE	---IDENTIFICATION---	----------RESULTS----------	COMMENTS
* 71 151 200001	01/29/82	RCCH SURFACE SWABS	#SWB =06		

SWAB#	BACTERIA	MOLD
1	2	0
2	0	1
3	8	0
4	3	0
5	2	0
6	2	0

MAINTENANCE INFORMATION FOR MICE

Item	Manufacturer or Supplier	City, State Address	Specifications	Frequency of Cleaning or Change
Cages	Lab Products, Inc., or Hazelton Systems, Inc.	Maywood, NJ Aberdeen, MD	Polycarbonate, 10 1/2" x 6 1/4" x 5"	1 X /week
Racks	Research Equipment	Bryan, TX	Stainless steel, 5 shelves, 69" high x 60 3/16" x 27 3/16"	1 X /week
Bedding	P.J. Murphy Forest Products Corp.	Rochelle Park, NJ	Hardwood chips, heat-treated	1 X /week
Cage Filters	Lab Products, Inc.	Maywood, NJ	Spun polyester	1 X /3 weeks
Room Air Filter	Flanders Filters, Inc. or American Air Filter	Washington, NC Louisville, KY	Industrial Grade HEPA Filter, 99.97% efficient at 0.3 µm	As required by air flow (about 1 X /year)
Feed	Ralston Purina Co.	St. Louis, MO	Autoclavable Meal 5010M	-
Feeders	Lab Products, Inc.	Maywood, NJ	Stainless steel wire grid, depression in cage cover	1 X /week
Watering System	Lab Products, Inc.	Maywood, NJ	Glass bottles, stainless steel sipper tubes, rubber stoppers	1 X /week
Rack Washer	Girton Mfg. Co.	Millville, PA	180°F wash & rinse (3 cycles)	-
Cage Washer	Girton Mfg. Co.	Millville, PA	180°F Tunnel Washer (4 cycles)	-
Cage & Rack Washing Compound	Dubois Chemicals	Cincinnati, OH	Mir-a-saf	-

NATIONAL CENTER FOR TOXICOLOGICAL RESEARCH

NTP/NCTR
EXPERIMENT: 05010 TEST: 02

RELATIVE TEMPERATURE AND HUMIDITY REPORT

TEST FROM 07/06/81 TO 10/15/81

DOXYLAMINE SENSOR_ID: 5A 151

REPORT: THRSUM01
DATE: 08/20/82
TIME: 14/51/14
PAGE: 1

TEMPERATURES OUT OF RANGE

H = HIGH L = LOW

DATE	TIME	TEMPERATURE	INDICATOR
M.A.		76.4	H
09JUL81	2118	78.4	H
09JUL81	2133	77.4	H
19SEP81	1103	75.1	H
19SEP81	1118	75.9	H
19SEP81	1133	76.4	H
19SEP81	1148	76.8	H
19SEP81	1203	77.2	H
19SEP81	1218	76.1	H
22SEP81	1115	76.2	H

NATIONAL CENTER FOR TOXICOLOGICAL RESEARCH

RELATIVE TEMPERATURE AND HUMIDITY REPORT

NTP/NCTR
EXPERIMENT: 05010 TEST: 02

TEST FROM 07/06/81 TO 10/15/81

DOXYLAMINE SENSOR_ID: 5A 151

REPORT: THRSUMC1
DATE: 08/20/82
TIME: 14/51/14
PAGE: 2

HUMIDITIES OUT OF RANGE

H = HIGH L = LOW

DATE	TIME	HUMIDITY	INDICATOR
09JUL81	0014	63	H
09JLL81	0029	60	H
09JUL81	1246	69	H
09JLL81	1301	68	H
09JUL81	1416	63	H
09JUL81	2018	74	H
M.A.		79	H
09JUL81	2048	68	H
09JLL81	2103	79	H
09JUL81	2118	79	H
09JLL81	2133	79	H
09JLL81	2148	73	H
09JLL81	2203	65	H
09JLL81	2218	63	H
10JUL81	1507	61	H
10JLL81	1522	63	H
24JUL81	1936	61	H
14AUG81	0838	60	H
14ALG81	0853	67	H

NATIONAL CENTER FOR TOXICOLOGICAL RESEARCH

RELATIVE TEMPERATURE AND HUMIDITY REPORT

TEST FROM 07/06/81 TO 10/15/81

NTP/NCTR
EXPERIMENT: 05010 TEST: 02

DOXYLAMINE SENSOR_ID: 5A 151

REPORT: THRSUM01
DATE: 08/20/82
TIME: 14/51/14
PAGE: 3

HUMIDITIES OUT OF RANGE

H = HIGH L = LOW

DATE	TIME	HUMIDITY	INDICATOR
22SEP81	1045	64	H
22SEP81	1100	71	H
22SEP81	1115	63	H
30SEP81	1144	64	H
30SEP81	1159	73	H
30SEP81	1214	68	H
30SEP81	1229	63	H

NATIONAL CENTER FOR TOXICOLOGICAL RESEARCH

RELATIVE TEMPERATURE AND HUMIDITY REPORT

TEST FROM 07/06/81 TO 10/15/81

NTP/NCTR
EXPERIMENT: 05010 TEST: 02

REPORT: THRSUMC1
DATE: 08/20/82
TIME: 14/51/14
PAGE: 4

DOXYLAMINE SENSOR_ID: 5A 151

TOTAL NORMAL TEMPERATURE TRANSACTIONS:8746

TOTAL HIGH TEMPERATURE TRANSACTIONS: 0010

TOTAL LOW TEMPERATURE TRANSACTIONS: 0000

AVERAGE TEMPERATURE: 71.2

TOTAL NORMAL HUMIDITY TRANSACTIONS: 8711

TOTAL HIGH HUMIDITY TRANSACTIONS: 0026

TOTAL LOW HUMIDITY TRANSACTIONS: 0000

NATIONAL CENTER FOR TOXICOLOGICAL RESEARCH

RELATIVE TEMPERATURE AND HUMIDITY REPORT

TEST FROM 07/06/81 TO 10/15/81

NTP/NCTR
EXPERIMENT: 05010 TEST: 02

DOXYLAMINE SENSOR_ID: 5A 151

REPORT: THRSUM01
DATE: 09/20/82
TIME: 14/51/14
PAGE: 5

MISSING TRANSACTIONS

T = TEMPERATURE H = HUMIDITY

TRANSACTION TYPE	FROM		TO	
T	1559	15JUL81	1752	15JUL81
H	1559	15JUL81	1752	15JUL81
T	0516	17JUL81	1900	18JUL81
H	0516	17JUL81	1900	18JUL81
T	1137	19JUL81	1424	19JUL81
H	1137	19JUL81	1424	19JUL81
T	1020	20JUL81	1743	20JUL81
H	1020	20JUL81	1743	20JUL81
T	1246	29JUL81	1417	29JUL81
H	1246	29JUL81	1417	29JUL81
T	0955	05AUG81	1110	05AUG81
H	0955	05AUG81	1110	05AUG81
T	1650	06AUG81	2311	06AUG81
H	1650	06AUG81	2311	06AUG81
T	0758	13AUG81	0944	13AUG81
T	1619	25AUG81	1906	25AUG81
H	1619	25AUG81	1906	25AUG81
T	0446	10SEP81	0646	10SEP81
H	0446	10SEP81	0646	10SEP81

NATIONAL CENTER FOR TOXICOLOGICAL RESEARCH

RELATIVE TEMPERATURE AND HUMIDITY REPORT

NTP/NCTR
EXPERIMENT: 05010 TEST: C2

TEST FROM 07/06/81 TO 10/15/81

DOXYLAMINE SENSOR_ID: 5A 151

REPORT: THRSUMC1
DATE: 08/20/82
TIME: 14/51/14
PAGE: 6

MISSING TRANSACTIONS

T = TEMPERATURE H = HUMIDITY

TRANSACTION TYPE	FROM		TO	
T	1347	12SEP81	1558	12SEP81
H	1347	12SEP81	1558	12SEP81
T	0011	25SEP81	0247	25SEP81
H	0011	25SEP81	0247	25SEP81
T	0347	25SEP81	0626	25SEP81
H	0347	25SEP81	0626	25SEP81
T	0603	06OCT81	0843	06OCT81
H	0603	06OCT81	0843	06OCT81
T	1009	06OCT81	1258	06OCT81
H	1009	06OCT81	1258	06OCT81
T	1913	15OCT81	2103	15OCT81
H	1913	15OCT81	2103	15OCT81

NATIONAL CENTER FOR TOXICOLOGICAL RESEARCH

FINAL REPORT

EXPERIMENT NO. 376, DOXYLAMINE, 90-DAY STUDY

IN $B6C3F_1$ MICE

APPENDIX C

TOXICOLOGICAL DATA

HEALTH AND HUMAN SERVICES
PUBLIC HEALTH SERVICE
FOOD AND DRUG ADMINISTRATION

NATIONAL CENTER FOR TOXICOLOGICAL RESEARCH
JEFFERSON, ARKANSAS 72079

OUTLINE OF THE NCTR PROTOCOL FOR THE NTP CARCINOGENESIS BIOASSAY OF

100% Pure [redacted]
7/1/81

DOXYLAMINE
PHASE III
90-DAY SUBCHRONIC STUDY

Approved: [redacted] Date: June 17 1981

Approved: [redacted] Date: June 24 81

Concur: [redacted] Date: 6-30-81

Concur: [redacted] Date: 6/24/81

FINAL
376
[redacted]
6-30-81
COPY

OUTLINE OF THE NCTR PROTOCOL FOR THE
NTP CARCINOGENESIS BIOASSAY OF

DOXYLAMINE

90-DAY SUBCHRONIC STUDY

2-[α-(2-dimethylaminoethoxy)-α-methylbenzyl]pyridine

CAS No. 46-92-16 NTP #195 NCTR # E-376
NTP #5010-02

Summary

Objective: To determine the potential pathological endpoints and estimate the maximum tolerated dose (MTD) in Fischer 344 rats and B6C3F1 mice when doxylamine is mixed into the diet. Results will be used to establish dose levels for a chronic study.

Route of Administration: Dosed feed

Rationale: This drug is taken orally as an antihistamine and as an over-the-counter sleep aid.

Chemical: Doxylamine succinate (dose calculated as base)

Animals: NCTR Fischer 344 rats and B6C3F1 mice, male and female, randomized by weight, ear marked and placed into treatment and control groups.

Absorption studies on test chemical not deemed necessary for this subchronic study.

PRECHRONIC STUDIES

NCTR #E-376
NTP #5010-02

Subchronic Test:

Purpose: This test provides the basis for setting dose levels for the chronic study. An indication of the type of pathological effects produced by the chemical will be obtained.

Treatment: At each of five (5) dose levels, twelve (12) animals per sex per species will be given doses of the chemical continuously in the feed until day 90 when dosed feed will be replaced by pelletized control feed until sacrifice on the following day. A fresh supply of dosed feed will be made available to the animals weekly when cages and bedding are changed. Twice daily checks will be performed and dead and moribund observations will be recorded. Clinical observations will be performed once a week.

Observations: (1) Activity-irritability; (2) lethargy-ataxia; (3) muscular twitching-spasms; (4) cyanosis; (5) apnea-hyperpnea (respiratory distress); (6) other clinical signs; (7) body weight and (8) death.

Test Groups:

Animals		Sex		Species		Dose levels		Total
12	x	2	x	2	x	5	=	240

Control:

Animals		Sex		Species		Total
12	x	2	x	2		48

Housing: Not more than three (3) rats and four (4) mice per cage; random assigned by weight and individual animals marked. Animals will be housed separately by sex, by species and by dose level.

Feed: Purina 5010 M

Water: Barrier water

Test Dosage: Rats (ppm, as doxylamine) 0, 162, 405, 1012, 2530, 6325
Mice (ppm, as doxylamine) 0, 80, 162, 325, 750, 1500
(Variance is ± 10%)

EXPERIMENTAL PROTOCOL

CAS No. 46-92-16 NTP #195 NCTR #E-376
NTP #5010-02

Title: Subchronic Toxicty Study on Doxylamine in Fischer 344 Rats and B6C3F1 Mice.

Principal Investigators: [redacted] NCTR, Jefferson, AR (541-4553)

Co-Principal Investigators: [redacted], NCTR, Jefferson, AR (541-4401)
[redacted] NCTR, Jefferson, AR (541-4519)

GENERAL

This study will be performed as the basic experimental protocol outlined in: Technical Protocol for the Bioassay of Doxylamine.

Approval for study to begin: [redacted] NTP Program Director for NCTR
Proposed start date of allocation: NA
Proposed start of test: 7/6/81 (Rats on 7/6/81; mice on 7/13/81)
Proposed end of test: Week of 10/12/81
Proposed submission date of report: Week of 12/14/81
Internal study ID(s): NCTR #E-376
Test Substance: Doxylamine succinate
Manufacturer: Richardson-Merrell, Inc.
Control substance: NA
Manfacturer: NA
Chemical reanalysis date(s) (study period only): July 1981

7/15/81 [redacted]

TEST SYSTEM

Source: NCTR

Species/Strain/Substrain (if applicable): Rat Fischer 344, Mouse B6C3F1

Sex: Male and Female

Age: 31 - 40 days

Acceptable range in body weights at start of test:

Species	Sex	Weight Range
Rat	Male	70 - 150 grams
Rat	Female	60 - 130 grams
Mouse	Male	13 - 26 grams
Mouse	Female	12 - 24 grams

Quarantine room #s: NA

Animal treatment room #: Bldg. 5A - 151

Procedure for individual animal identification: All animals will be identified by an ear clip system and by cage cards which will include experiment number, cage number, room number, dose code, rack number, start date and treatment number.

Randomization table used: [redacted] memo (attached)

EXPERIMENTAL DESIGN

Route of administration: Dosed feed

Frequency of administration: Ad libitum

Dose levels: Rats (ppm, as doxylamine) 0, 162, 405, 1012, 2530, 6325

Mice (ppm, as doxylamine) 0, 80, 162, 325, 750, 1500

(Variance is ± 10%)

Protocol No.: NCTR #E-376

No. treated animals required/dose level: 12/sex/species

No. vehicle control animals required:

No. untreated control animals required: 12/sex/species

Feed: Purina 5010 M

Frequency: Ad Libitum

Contaminants in feed expected to interfere with study: none known, however analysis performed at NCTR[1].

Water: Barrier water

Frequency: Ad libitum; from bottles

Contaminants in water expected to interfere with study: none known, periodic water analysis performed at NCTR

Weighing Schedule: Animals will be weighed individually at the initiation, after each week, and at the termination of the test. Feed will be weighed initially, after each week, and at the termination of the test.

Date(s) of and number of animals required for collection of plasma samples for the animal disease screening program: none required

Statistical analysis to be performed: Determination of maximum tolerated dose (see attachment).

Comments:

Rats (3/cage) material of cage: Polycarbonate

Mice (3/cage) material of cage: Polycarbonate

Rat cage measure: 17" x 8 1/4" x 8"

Mice cage measure: 10 1/2" x 6 1/4" x 5"

Animal bedding: Hardwood chips-P. J. Murphy Forest Products Corp.
114 Essex St.
Rochelle Park, NJ 07662

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[1]Policies and Procedures for Murine Rodent Diets-January, 1981, Food and Drug Administration, National Center for Toxicological Research, Jefferson, AR

DOSAGE MIXING, HANDLING AND STORAGE

Chemical name: Doxylamine

Date of first mixing: Week of 6/29/81

Frequency of mixing: Twice

Decontamination and/or cleanup procedure: As per NCTR Safety Guideline (attached)

The following information must be provided below: materials required, area of performance, safety requirements, mixing procedure, storage and use of the mixed chemical.

Material required:

1. Dosage preparation information form
2. Balance
3. Stock solutions of doxylamine
4. Gloves
5. Mask

Area of performance and safety requirements: All mixing procedures will be handled in diet preparation facility, the technician will be protected by gloves and mask.

Mixing procedures:

1. During all procedures, protect the chemical from light and air as much as possible.
2. Details of the mixing procedure are attached.
3. Doxylamine succinate will be weighed and dissolved in ethanol in a volumetric flask. Concentrations of stock solutions will be guaranteed by weight (QA Inspector).
3. Chemistry will certify the stock solutions concentration of doxylamine in each initial batch of feed prior to its use in the study, will determine the concentration of one other batch at each dose level during the course of the study and will determine stability of the compound mixed with the feed at the highest and lowest dose levels.

Handling, storage and use: Doxylamine is stable in feed for at least eight weeks.

Pathology:

1. A routine necropsy will be done as indicated in the Pathology Services Standard Operating Procedures. This will include weighing the animal.
2. Weigh liver.
3. Fix and process the following organs and any gross lesions for microscopic evaluation: liver, lung, right kidney, heart, thymus, brain, stomach and target organs as requested by PI. Other organs will be discarded.
4. All fixed tissues will be examined histologically.
5. Special sections of the liver will be stained for lipid.
6. Gross and microscopic pathology reports will be submitted to the Division of TDMS for entering on the data base.

Records to be maintained/Reports to be generated:

1. Automated:
   a. Animal weights and observations
   b. Animal removal reports for dead or scheduled sacrifices
   c. Gross pathology
   d. Microscopic pathology
   e. Environmental condition and control data, JC-80
   f. Weight anomalies
   g. Missed cages
   h. First observation for animals on experiment.
   i. Liver weight summaries, including absolute liver weight and liver/body weight ratios.
2. Manual:
   a. Chemical purity and stability
   b. Chemical certification of concentration
   c. Allocation of animals and animal allocation weights
   d. Feed/water mixing sheets

If automated system breaks down, manual system will be substituted for each category.

ATTACHMENTS

1. Suggested Randomization and Rack Configuration for NTP Fourteen Day Repeated Dose Study.

2. Start-up Information for NCTR Experiment #E-376

3. Safety Guidelines

4. Pathology Protocol

5. Determination of Maximum Tolerated Dose (MTD)

SAFETY GUIDELINES

Test Article(s): Antihistamines

Test Article(s): Doxylamine
Mol. Wt. 270.38

Synonyms: N-N-dimethyl-2-[1-phenyl-1-(2-pyridinyl)ethoxy]ethamamine; 2[alpha-(2-dimethylaminoethoxy)-alpha-methylbenzyl]pyridine.

Characteristics: Liquid at room temperature, boiling point 137-141° C. at 0.5 mm Hg.

Test Article(s): Doxylamine Succinate NF
Mol. Wt. 388.46
CAS No. 526-10-7
NIOSH No. US 92750

Synonyms: N,N-dimethyl-2-[1-phenyl-1(2-pyridinyl)ethoxy]-ethanamine-butanedioate(1:1); 2(alpha-(2-dimethylamino)ethoxy-alphamethylbenzyl)-pyridine succinate (1:1) (NIOSH listing); 2-[α-[2-(Dimethylamino)ethoxy]-α-methylbenzyl]-pyridine succinate (1:1); Decapryn Succinate.

Characteristics: White or creamy white powder with characteristic odor; melts with 3° range between 103° and 108° C.; soluble in water, alcohol and chloroform. Slightly soluble in ether and benzene.

Test Article(s): Methapyrilene
Mol. wt. 261.38
CAS No. 91-80-5
NIOSH No. UT 14000

Synonyms: 2-((2-(dimethyamino)ethyl)-2-thenylamino)-pyridine (NIOSH listing); N-N-dimethyl-N'-2-pyridinyl-N'(2-thienylmethyl)-1,1-ethanediame

Characteristics: Liquid at room temperature; boiling point 125-135° C. at 0.45 mm Hg, 173-175° C. at 3 mm Hg. Specific gravity 1.5835 or 1.5842.

Test Article(s): Methapyrilene Fumarate NF
Mol. wt. 870.99
CAS No. 33032-12-1

Synonyms: 2-[[2-(dimethylamino)ethyl]-2-thenylamino]pyridine fumarate (2:3); 1,2-ethandiame, N, N-dimethyl-N'-2-pyridinyl-N'-(2-thienylmethyl)-(E)-2-butenedioate (2:3)

NTP Experiment

Characteristics: White, crystalline powder, usually having a faint odor; melting point between 133° and 137° C.; soluble in water and in alcohol; a solution of 1 gm in 20 ml has pH of 3-4.

Test Article(s): Methapyrilene hydrochloride NF
Mol. wt. 297.85
CAS No. 135-23-9
NIOSH no. UT 17500

Synonyms: 2-[[2-diethylamino)ethyl]-2-thenylamino]pyridine monohydrochloride (NIOSH listing); 1,2-ethanediamine, N, N-dimethyl-N'-2 pyridinyl-2'-(2-thienyl-methyl)-monohydrochloride; Histadyl

Characteristics: White, crystalline powder, usually having a faint odor, has bitter taste; melting point 161-165° C. One gram dissolves in about 0.5 ml water, about 5 ml alcohol, and about 3 ml chloroform. Practically insoluble in benzene and ether. Water solutions have a pH of about 5.5

Test Article(s): Pyrilamine
Mol. wt. 285.38
CAS No. 91-84-9
NIOSH No. UT 08750

Synonyms: 2-((2-dimethylamino)ethyl)(p-methoxybenzyl)amino)-pyridine (NIOSH listing); 1,2-ethanediqmine, N-[(4-methoxypheny)methyl]-N', N'-dimethyl-N-2-pyridine; Wait's Green Mountain Antihistamine.

Characteristics: Oily liquid at room temperature; boiling point 168-172° C. at 0.06 mm Hg, 201° C. at 5 mm Hq.

Test Article(s): Pyrilamine hydrochloride
Mol. wt. 321-89
CAS No. 6036-95-9
NIOSH No. UT 10500

Synonyms: 2-((2-dimethyamino)ethyl)(p-methoxybenzyl)amino)-pyridine hydrochloride (NIOSH listing); Mepyramine hydrochloride.

Characteristics: Crystals; melting point 143-143.5° C.; very soluble in water.

Test Article(s): Pyrilamine maleate NF
Mol. wt. 401.46
CAS No. 59-33-6
NIOSH No. UT 12250

Synonyms: 2-((2-(dimethylamino)ethyl)(p-methoxybenzyl)amino)-pyridine maleate (1:1) (NIOSH listing); 1,2-ethanediamine, N-[(4-methoxyphenyl)methyl]-N', N'-dimethyl-N-2-pyridinyl-(Z)-2-butenedioate (1:1); Antallergan Maleate.

Characteristics: White, crystalline powder, usually having a faint odor; bitter saline taste; melting point 99-103° C. One gram is soluble in about 0.5 ml water, 15 ml absolute alcohol, about 2 ml chloroform. Slightly soluble in benzene and ether. A 10% solution has a pH of about 5.1. Raising the pH of aqueous solutions to 7.5-8.0 allows release of the oily freebase which is heavier than water. The dry powder is stable in air.

Test Article(s): Tripelennamine
Mol. wt. 255.35
CAS No. 91-81-6
NIOSH No. US 28000

Synonyms: 2(benzyl(2-(dimethylamino)ethyl)amino)-pyridine (NIOSH listing); N,-N-dimethyl-N'-(phenylmethyl)-N'-2-pyridinyl-1,2-ethanediamine; tripelenamine.

Characteristics: Oily liquid with amine odor; boiling point 167-172° C. at 0.1 mm Hg, 185-190° C. at 1.7 mm Hg. Miscible with water.

Test Article(s): Tripelennamine hydrochloride USP
Mol. wt. 291.82
CAS No. 154-69-8
NIOSH No. US 31500

Synonyms: 2-(benzyl(2-(dimethylamino)ethyl) pyridine hydrochloride (NIOSH listing); Pyribenzamine; Resistamine.

Characteristics: White, crystalline powder, with bitter taste, producing temporary numbness of the tongue; melting range 188-193.5° C.; one gram dissolves in about 1 ml water, about 6 ml alcohol, about 6 ml chloroform, about 350 ml acetone. Practically insoluble in benzene, ether and ethyl acetate. The powder darkens slowly on exposure to light. Aqueous solutions have pH as follows: 25 mg/ml, 6.71; 50 mg/ml, 6.67; 100 mg/ml 5.56.

Test Article(s): Tripelennamine citrate USP
Mol. wt. 447.49
CAS No. 6138-56-3

Synonyms: 1,2-ethanediamine, N, N,-dimethyl-N'-(phenylmethyl)-N'-2-pyridinyl-2-hydroxy-1,2,3-propanetricarboxylate (1:1); 2-[benzyl[2-(dimethylamino)ethyl]amino]-pyridine citrate (1:1).

Characteristics: White, crystalline powder; melting range 106-110° C. One gram dissolves in about 1 ml water; freely soluble in alcohol; very slightly soluble in ether; practically insouble in benzene or chloroform. A 1% aqueous solution has a pH of 4.25. Taste less bitter, more palatable, than hydrochloride.

Test Article(s): Thenyldiamine
Mol. wt. 261.36
CAS No. 91-79-2
NIOSH No. UT 15750

Synonyms: 2-((2-(dimethylamino)ethyl)-3-thenylamino)-pyridine (NIOSH listing); N, N-dimethyl-N'-2-pyridinyl-N'-(3-thienyl-methyl)-1, 2-ethanediamine; Thendadil

Characteristics: Liquid at room temperature; boiling point 169-172° C. at 1.0 mm Hg.

Test Article(s): Thenyldiamine hydrochloride (mono)
Mol. wt. 297.88
CAS No. 958-93-0
NIOSH No. UT 19250

Synonyms: 2-((2-(dimethylamino)ethyl)-3-thenylamino)-pyridine mono hydrochloride (NIOSH listing);
N-N-dimethyl-N'-(3-thenyl)-N'-(2-pyridyl) ethylene diamine hydrochloride.

Characteristics: Crystalline solid at room temperature; melting point 169.5-170° C.; bitter taste; soluble in water to 20%; slightly soluble in alcohol; pH of 1% aqueous solution 6.5.

Test Article(s): Chlorothen
Mol. wt. 295.85, 295.86
CAS No. 148-65-2
NIOSH No. US 73500

Synonyms: 2-((5 chloro-2-thenyl)(2-(dimethylamino)ethyl)amino)pyridine (NIOSH listing); N-((5-chloro-2-thienyl)methyl)-N',N'-dimethyl-chloro-2-pyridinyl-1,2-ethanediamine.

Characteristics: Liquid at room temperature. Boiling point 155 - 156° C. at 1.0 mm mercury. Strong base.

Test Article(s): Chlorothon citrate NF XIII
Mol. wt. 487.95
CAS No. 148-64-1

Synonyms: (2-((5-chloro-2 thenyl)[2-dimethylamino)-ethyl)amino) pyridine citrate (1:1); Chloromethapyrilene citrate; Tagathen.

Characteristics: White, crystalline powder, with faint odor. Melting point 112 - 116° C. On further heating, gradually solidifies and remelts at 125 - 140° C., with decomposition. One gram dissolves in 35 ml water, 65 ml EtOH. Practically insoluble in benzene, chloroform, diethyl ether. Aqueous solutions are acid to litmus, pH of 1% solution, 3.9 - 4.1.

Test Article(s): Chlorothen hydrochloride
Mol. wt. 332.32
CAS No. 135-35-2
NIOSH No. US 74000

Synonym: Tagathen

Characteristics: Crystal at room temperature. Melting point 106 - 108° C. Freely soluble in water.

Assay Methodology: Chemistry Division at NCTR is currently validating a method for the quantitative and qualitative determination of the antihistamines, using gas chromatography with a nitrogen-phosphorus detector.

Human Health Effects: Each of these test articles may be classified as an antihistaminic, or an antihistaminic drug. The indications for the use of the various antihistaminic drugs vary considerably. The majority of these drugs are effective in perennial and seasonal allergic rhinitis, vasomotor rhinitis, allergic conjunctivitis, uticaria, and angioedema, allergic reactions to blood and plasma, dermographism, and as adjuncts to conventional therapy in anaphylactic reactions. Some of these antihistamines are effective in the active and prophylactic management of motion sickness. The more sedative agents are sometimes used as substitutes for the barbituates in insomnia, and insomnia predominant in certain medical disorders. Certain of the antihistaminics are used in propietary medications widely promoted for insomnia.

It is generally agreed that most antihistamine drugs are ineffective in migraine and histamine headache prevention, or regulation of the sequela of pain, edema, and hemorrhage in oral surgery, potentiation of narcotic analgesic drugs, as antiemetic agents in post-operative patients, as antitussive agents, or for treatment of nocturnal leg cramps, leg cramps of pregnancy and functional dysmenorrhea. The usefulness of antihistaminic drugs in various other clinical conditions such as bronchial asthma, atopic dermatitis, neurodermatitis, allergic eczema, various contact and chemotoxic dermatitides, generalized pruritis, use in cardiac arrhythmias, spasmolysis

In gastrointestinal allergies, prophylaxis of drug reactions, etc. must await further clinical investigation before a final assessment can be made.

The antihistamines produce a variety of undesirable side effects. Although their incidence varies considerably, ranging from less than 7% to as high as 80%, they are similar for all the available agents. The drugs may even produce sensitivity reactions of the type which they usually inhibit. Sensitivity to one antihistamine is ordinarily not an indication that the patient is sensitive to others.

Sedation is the most common untoward reaction and is directly related to the dosage employed. The reaction is particularly serious when the drug is taken without adequate medical supervision. In some cases, this response may disappear after several days of medication. Other side reactions elicited by these drugs include nausea, headache, vertigo, xerostomia, restiveness, and gastrointestinal distress. Dermatological complications and skin eruptions have followed the local application or oral administration of antihistamines. In a few individuals, certain antihistaminics produce signs of central excitation, such as insomnia and nervousness. Normal dose for humans is 100 to 200 mg/day, usually divided into four doses.

Employees conducting research with antihistamines at NCTR are unlikely to be exposed to amounts anywhere approaching therapeutic doses. Therefore, the effects that have been noted in patients receiving the drugs for therapeutic purposes are unlikely to be experienced among employees. However, since evidence is present in the literature that antihistaminics can produce local sensitivity, care to avoid skin contact is mandatory when research is conducted at NCTR with these drugs.

Toxicity: Antihistamines are effective in preventing histamine shock in guinea pigs, bronchospasm induced in guinea pigs by nebulized histamine solutions, whealing on the skin, and many other responses to histamine. The hypotension induced by histamine is more difficult to block, and the increased salivation and gastric secretion are not inhibited. The antihistamines also have antianaphylactic properties in large doses, and are antipruritic and analgesic. Some have bowel or bladder smooth muscle antispasmodic action, and some produce sedation and other central nervous system stimulation. Strong evidence is present in the literature that methapyrilimine produces tumors in laboratory test animals. There is no evidence in the literature that other antihistamines produce a similar effect in laboratory animals.

The antihistamines are not particularly toxic to laboratory test animals, the LD_{50} by the oral route for mice ranging from about 182 mg/kg body weight to about 470 mg/kg body weight.

Emergency Treatment and Medical Surveillance: Because of the characteristics of these antihistamines, it is likely that the organic acid esters will be used in research at NCTR. Alkaline pH's may cause the esters to separate, releasing the free oily amine base. If the substance comes into contact with unprotected skin, immediately wash the affected area with clean, flowing water for 10 minutes. If there is evidence of an oily residue on the skin following this emergency treatment, continue to wash the skin area, using soap and water. Soap and water should help remove the oily amine base; however, using soap (with its alkaline pH) may cause the ester form of the antihistamines to break down, which would make the removal problem worse. Therefore, it is recommended that water alone first be used to flush skin areas before using soap.

For eye exposure, the eyes should be flushed with clean, flowing water for 10 minutes, as one would following any eye exposure to chemicals.

In case of ingestion, no special action is indicated, because the amount likely to be ingested during research at NCTR is far less than therapeutic doses.

In cases of any exposure to antihistamines the employee should notify the supervisor of the exposure and then report to the NCTR Occupational Health Unit for any followup care or treatment which might be indicated, depending upon the symptoms produced.

Protective Measures: Research with pure antihistamines should be conducted in compliance with the NCTR Safety Manual, Section VI.B., Identification and Control of Hazardous Substances Use Areas, page VI-2; and VI.E., Hazardous Substances Control and Containment, page VI-21.

Employees who have demonstrated that they can safely handle antihistamines may conduct research on the open bench, when the quantity of the substance is less than 1 gram. The employees' supervisors and the Principal Investigator should agree to any deviations from this open-bench provision, and this agreement will be documented.

When the research being conducted involves treating laboratory animals with antihistamines, and when the substance is administered via the feed or water, it will not be necessary to document the open-handling provision for animal caretakers working in animal rooms or in support areas where cages, etc. are cleaned.

When moved from one place to another, antihistamines should be contained as described in the NCTR Safety Manual, Section VI.C., Facilities Operation, page VI-6.

Employees must wash their hands before leaving rooms where antihistamines are used and before eating, smoking, etc.

Protective Clothing: Employees conducting research using antihistamines must be provided protective clothing in compliance with the NCTR Safety Manual, Section VI.D., Protective Clothing, Protective Devices, Showers and Change Room Practices, page VI-11. Deviations from this procedure must be authorized by the lab supervisor and the justification documented.

Occupational exposure to antihistamines may result in sensitization to the specific antihistamine. Should sensitization develop, the employee may not be able to use over-the-counter preparations which are useful in treating a variety of common illnesses.

To prevent skin contact with antihistamines, employees will wear impervious gloves, such as rubber surgical gloves, plastic examination gloves, etc. If the work involves heavy articles, or if there are sharp edges which might puncture or tear thinner gloves, supervisors should consider providing employees with heavy-duty rubber or plastic gloves.

Supervisors are responsible for training employees to properly wear protective clothing.

To prevent inhaling antihistamines, a respirator providing protection equivalent to the 3M 8710 or the 3M 9910 will be provided. Some of the antihistamines may have a mild characteristic odor, which does not reflect a hazard to individual health. However, if employees find this odor objectionable, the 3M 8712 or 3M 8713, or respirators providing equivalent protection may be used. Equivalent protection is demonstrated by NIOSH certification. To prevent the vapor removing capacity from being exhausted, the respirator should be sealed from the environment until ready to be used.

Supervisors are responsible for training employees to properly fit and to correctly wear respiratory protective equipment. Correct use of a respirator will effectively prevent exposure by ingestion.

Employees performing tasks where there is a chance that liquids containing antihistamines may splash on their faces will be provided with a face shield which affords full-face protection.

Employees will wash their hands after removing protective clothing.

Decontamination: Laboratory glassware, and other equipment used during the conduct of research with antihistamines, will be decontaminated following procedures described in the NCTR Safety Manual, Section VI.G. (Detoxification and Decontamination, page VI-35). Water may be used as the preliminary rinse solvent for the ester forms of the antihistamines. Acetone should be used to rinse glassware contaminated with the free base amine form of the antihistamines. Water used as a preliminary rinse solvent may be dumped down the drain. Acetone used as a preliminary rinse solvent must be collected and disposed of as waste solvent.

Waste Disposal: The procedures for handling waste products found in the NCTR Safety Manual, Section VI.H. (Handling of Hazardous Laboratory and Animal Waste, page VI-41) are adequate for waste material containing antihistamines.

Spill Decontamination: Spill cleanup involves physical removal of the antihistamine, together with the carrier or vehicle used to administer the substance. Antihistamines in the ester form are quite water soluble, so that washing with water will be quite effective. Using a surfactant (with its alkaline pH) when the antihistamine is present in the form of the ester may cause the release of the oily free amine base. For this reason, it is important to know the composition of the antihistamine being used in a specific experiment, so that one may anticipate the proper spill cleanup procedure. In the event the antihistamine is in the free base form, the use of a good surfactant (such as Microquat or Hyamine) will make cleanup more effective. When using water or water with surfactants, the material used for spill cleanup may be flushed down the nearest floor drain. In the event no floor drain is available, the spill cleanup materials must be collected and disposed of in accordance with the NCTR Safety Manual, Section VI.H., Handling of Hazardous Laboratory and Animal Waste, page VI-41.

A report of the event, containing the data outlined in the NCTR Safety Manual, Section IV.B. (Accidental Exposure to Hazardous Substances, page IV-1) will be prepared by the supervisor, with copies sent to the NCTR Occupational Health Unit, the Principal Investigator, the QAS office and the Safety Officer.

Workplace Monitoring: Workplace monitoring is accomplished by wiping cotton-tipped swabs over the surface. The cotton-tipped swabs usually are moistened using a suitable solvent before use. The selection of solvent must await the validation of analytical methods for antihistamines by the Chemistry Division, NCTR.

MEMORANDUM

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

TO : [redacted]
Project Manager, NTP Chemical Evaluation

DATE: May 5, 1981

FROM : [redacted]
Dir., Div. of Biometry

SUBJECT: Randomized Allocation and Rack Configuration for NTP Subchronic Studies

INTRODUCTION

The 13-week NTP subchronic experiment is conducted in both sexes of Fischer 344 rats and B6C3 mice. Six dosage levels (including controls) with 12 animals per dosage level are used. Rats are housed 3 per cage and mice are housed 4 per cage. The total number of animals is:

72	male rats
72	female rats
72	male mice
72	female mice
288	

The 13-week pathology, mortality, and animal weight are used to estimate the maximum tolerated dose (MTD) for the two year chronic studies. Because of the pathology workload capacity, not all animals will be necropsied after exactly 13 weeks. Thus, the 13-week animal room body weight of an animal will be used rather than the body weight obtained at necropsy.

RANDOM ALLOCATION

The random allocation of animals to cages on a weight basis is accomplished as follows for any particular species and sex. Weigh the animals and arrange in order from lightest to heaviest. The allocation begins with the lighter animals and proceeds through the heavier animals. The automated allocation scheme based on computer generated randomized cage assignments may be used. This will provide a range of lighter to heavier animals in each cage.

MALE RATS RACK CONFIGURATION

The rack configuration for male rats is given in Figure 1 showing cage numbers and dosage levels. The top shelf is not used. The six dosage levels are designated by 1-6 with 1 being the controls up to 6 being the heaviest dosage level. Two rat racks per sex are utilized in order to provide 6 columns of cages. All of the 4 cages in a column receive the same dosage level. The dosage levels have been assigned to columns at random.

FIGURE 1. Male Rats Rack Configuration (Cage # - Dose Level #)

-	-	-	-
1 - 3	5 - 1	9 - 5	-
2 - 3	6 - 1	10 - 5	-
3 - 3	7 - 1	11 - 5	-
4 - 3	8 - 1	12 - 5	-

-	-	-	-
13 - 2	17 - 6	21 - 4	-
14 - 2	18 - 6	22 - 4	
15 - 2	19 - 6	23 - 4	
16 - 2	20 - 6	24 - 4	

FEMALE RATS RACK CONFIGURATION

The rack configuration for female rats is given in Figure 2 showing cage numbers and dosage levels. The six dosage levels are designated by 1 for the controls and 7 - 11 for the chemical. The chemical dosage levels may or may not be the same as those used for the male rats.

FIGURE 2. Female Rats Rack Configuration (Cage # - Dose Level #)

-	-	-	-
25 - 9	29 - 11	33 - 7	-
26 - 9	30 - 11	34 - 7	-
27 - 9	31 - 11	35 - 7	-
28 - 9	32 - 11	36 - 7	-

-	-	-	-
37 - 8	41 - 1	45 - 10	-
38 - 8	42 - 1	46 - 10	-
39 - 8	43 - 1	47 - 10	-
40 - 8	44 - 1	48 - 10	-

MALE MICE RACK CONFIGURATION

The rack configuration for male mice is given in Figure 3 showing cage numbers and dosage levels. Animals are housed 4 per cage with 3 cages per dosage level on one side of a mouse rack. The 3 cages in a column receive the same dosage level. The dosage levels have been assigned to columns at random. The six dosage levels are designated by 1 for the controls and 12 - 16 for the chemical dosages.

FIGURE 3. Male Mice Rack Configuration: Side A
(Cage # - Dosage Level #)

49 - 13	52 - 15	55 - 1	58 - 16	61 - 12	64 - 14
50 - 13	53 - 15	56 - 1	59 - 16	62 - 12	65 - 14
51 - 13	54 - 15	57 - 1	60 - 16	63 - 12	66 - 14

FEMALE MICE RACK CONFIGURATION

The rack configuration for female mice is shown in Figure 4 giving cage numbers and dosage levels. The females are housed on the opposite side of the rack from the males. The six dosage levels are designated by 1 for the controls and 17 - 21 for the chemical. The chemical dosages for the females may or may not be the same as for the males.

FIGURE 4. Female Mice Rack Configuration: Side B
(Cage # - Dosage Level #)

-	-	-	-	-	-
67 - 18	70 - 20	73 - 21	76 - 1	79 - 19	82 - 17
68 - 18	71 - 20	74 - 21	77 - 1	80 - 19	83 - 17
69 - 18	72 - 20	75 - 21	78 - 1	81 - 19	84 - 17
-	-	-	-	-	-
-	-	-	-	-	-

START-UP INFORMATION FOR NCTR EXPERIMENT 376

NTP EXPERIMENT 5010-02

ROOM 151 A-BARRIER

"90 DAY - SUBCHRONIC-DOXYLAMINE"

ANIMALS:

Fischer 344 Rats	72 males and 72 females
B6C3F1 Hybrid Mice	72 males and 72 females

ALLOCATION:

Rats are to be allocated 3 per cage and ear marked N, L, and R on July 2, 1981.
Mice are to be allocated 4per cage and ear marked B, N, L, and R on July 8, 1981.
The rats and mice are to be given AW feed at time of allocation in order to get adjusted to the feeder.

All animals are to receive water from the A-Barrier water supply.

WEIGHTS, OBSERVATIONS, AND CAGE CHANGE SCHEDULE:

See attached schedule.

The technicians are to utilize the Clinical Observation Table provided for clarification of abbreviations.

FOOD AND FOOD CONSUMPTION:

The animals are to be fed AW control feed until put on dose.

See attached schedule.

Weekly food consumption is to be measured.

DATA COLLECTION:

All data entries are to be collected via:

1) EIS Terminal

2) Manual Form - See form attached.

3) Removal of Animals - See animal removals.

ANIMAL REMOVALS:

Unscheduled - Dead and Moribund animals are to be removed as follows:

1. Remove as "DEAD" and "MORIBUND" utilizing the EIS terminal or the EIS manual form provided.
2. Fill out an EDCS "Single Animal Removal" form as well.

Scheduled - Remove as "terminal":

1. Use EIS terminal for the animal removal.
2. Assign CID's in the following order: N,L, and R.
3. Write earclip on back of CID cards and wrap them to cage card.
4. Take to Pathology.
5. No EDCS single animal removal form is needed.

CID CARDS:

The CID cards for this experiment have been issued in two blocks.

#'s 1-144 have been designated for RATS and have RATS marked the cards.

#'s 145-288 have been designated for MICE.

All animals are to be taken to Pathology.

DIAGNOSTIC ANIMALS:

None.

ENVIRONMENTAL MONITORING:

1. Environmental Test

Date	Day	Bedding & Waste 5 cages at random	Water All bottles	Air	Specie	Rac
7-8-81	Wednesday			X		
7-9-81	Thursday	X	X		Rats	1-4
8-5-81	Wednesday	X	X	X	Mice	5
9-2-81	Wednesday			X		
9-3-81	Thursday	X	X		Rats	1-4
10-7-81	Wednesday	X	X	X	Mice	5

2. Chemistry Feed Samples

QA samples are to be collected from spares during the study when scheduled by the Chemistry Department.

DOSING OF ANIMALS:

See separate information.

[redacted]
Principal Investigator

June 29, 1981
Date

[redacted]
Program Resources, Inc.

June 29, 81
Date

BASIC PROGRAM NTP SUB-CHRONIC STUDIES "Doxylamine"

General daily activities: A.M. and P.M. death check.
Tap feeders during P.M. death check.

NCTR Expt. # 376
NTP # 5010-02

DATE	DAY	ALLOCATION		ON DOSE		W/O + CC		CC RATS	WK. ON DOSED FEED	
		RATS	MICE	RATS	MICE	RATS	MICE		RATS	MICE
7-2-81	Th	X								
7-6-81	M			X		X				
7-8-81	W		X							
7-9-81	Th							X		
7-13-81	M					X			1	
7-15-81	W				X		X			
7-16-81	Th							X		
7-20-81	M					X			2	
7-22-81	W						X			1
7-23-81	Th							X		
7-27-81	M					X			3	
7-29-81	W						X			2
7-30-81	Th							X		
8-3-81	M					X			4	
8-5-81	W						X			3
8-6-81	Th							X		
8-10-81	M					X			5	
8-12-81	W						X			4
8-13-81	Th							X		
8-17-81	M					X			6	
8-19-81	W						X			5
8-20-81	Th							X		
8-24-81	M					X			7	
8-26-81	W						X			6
8-27-81	Th							X		
8-31-81	M					X			8	
9-2-81	W						X			7
9-3-81	Th							X		
9-7-81	M	Holiday				X			9	
9-9-81	W						X			8
9-10-81	Th							X		
9-14-81	M					X			10	
9-16-81	W						X			9
9-17-81	Th							X		
9-21-81	M					X			11	
9-23-81	W						X			10
9-24-81	Th							X		
9-28-81	M					X			12	
9-30-81	W						X			11
10-1-81	Th							X		
10-5-81	M					X			13	
10-7-81	W						X			12
10-8-81	Th									
10-12-81	M									
10-14-81	W						X			13

Rats and mice are to be put on pelleted feed twenty-four (24) hours before removal and fast- overnight before removal to Pathology. Principal Investigator is to provide separate removal

*M-Th may be substituted with T-Fri. schedule.

PATHOLOGY PROTOCOL

Replaced Previous Path. Protocol.

Date Prepared July 14, 1981

Signed: [redacted] (Principal Investigator) Signed: [redacted] (Assigned Pathologist)

Experiment No. 376 Title: Doxylamine Subchronic

Compound Used Doxylamine Total Animals 288 Sex M & F

Strain Fisher 344 (Rats); B6C3F1 (Mice) Load Date July 1981 Completion

CID's 376-00001 thru 00144; 00145 thru 00288

Estimated Pathology Technician Man Years

Estimated Pathology Professional Man Years

To be filled in by PSP

RECEIVING

Data Terminal

x Used ___ Not Used

CLINICAL PATHOLOGY T.B.A.

Blood Smear: ___ Collection Method: Orbital ___ Other (describe)

Clin. Chem. Test Required:

Urinalysis:

Hematology:

HISTOPATHOLOGY

Organs to be Weighed

X Liver X Brain X Heart X Lungs ___ Spleen X R.Kidney ___ L.Kidney

X Thymus ___ Uterus ___ Pituitary X R.Testis ___ L.Testis ___ Ovaries ___ Adrenals

Organs to be shared (List organs and division)

Other Organs (List)

Revised March 17, 1980

___Routine	___Specific Organs	___Coil Ureter	___Vaginal Smear	___Bladder Inflat.
___Tra/Tyd/Esog	___Kdy/Adrl	___Tongue	___Sple/Pan	___Lymp Node
___Hrt/Tym/Aort	___Sternum	___Stomach	___Subdib	___Penis
___Ovary/Utrs	___Eye/Hrdan	___Duodenum	___Sublin	___Mammary
___Vag/Cervx	___Zymbal(Rat)	___Jejum	___Parotid	___Nasal Septum
___Testis/Fpi	___Muscle	___Ileum	___Lacrml	___Ext. Ear Canal
___Preputial	___Ureter	___Colon	___Crebrum	___Mammary WM*
___Sem.V/Coag	___Urethra	___Liv/GB	___Crebellum	___Intes. Exam*
___Bldr/Prost	___Bladder	___Lung	___Spinal Cord	

Other (Specify) _____

_____

Fixative: Routine X Other (name fixative & tissue) _____

_____

Trimming: Routine X Other _____

_____

HISTOTECHNOLOGY

Processing: Mice (4 hrs) X Other Rats 16 hrs

_____(16 hrs)

Embedding: Routine X Other _____

_____

Sectioning: Routine X Other _____

_____

Stain: Routine X Special _____

_____

_____

Data Liaison: Routine X Other _____

_____

* These methods will be done only through the coordination of a Pathologist.

NTP NECROPSY

(From BOA Draft Feb. 5, 1981, pp. 35.5 and 41.3)

Complete necropsy and fixation of following organs:

Inflate bladder and lungs of all animals

1 Gross Lesions
2 Skin (CX)
1 Mandibular Lymph Node (LX)
1 Mammary Glands (LX)
1 Salivary Glands (LX)
2 Thigh Muscle
2 Sciatic Nerve (CX)
1 Sternum (LX)
2 Costochondal Rib Junction (LX)
2 Oral Cavity
2 Larynx
2 Pharynx (CX)
1 Trachea (CX)
1 Esophagus (CX)
1 Thyroid/Parathyroids (CX)
1 Lungs/Bronchi(LX)
1 Heart/Aorta (LX)
1 Thymus (LX)
1 Stomach/Duodenum (LX)
1 Jejum (CX)
1 Ileum (CX)
2 Cecum (CX)
1 Colon (CX)
2 Tongue (CX)
1 Liver (Median, Left and Right)
1 Gall Bladder (Mouse Only)
1 Spleen (LX)
1 Pancreas (LX)
1 Kidneys (LX)
1 Adreanals (CX)
1 Urinary Bladder (LX)
2 Seminal Vesicles
1 Prostate (CX)
1 Testes (CX)
2 Tunica Vaginalis Testis
2 Scrotal Sac
1 Ovaries (CX)
1 Uterus (CX)
1 Nasal Cavity & Trubinates (CX)
1 Brain (Frontal Cortex Basal Ganglia
Parietal Cortex/Thalamus
Cerebellum/Pons) (CX)
1 Pituitary (CX)
2 Spinal Cord (CX)
2 Eyes (CX)
1 Preputial or Clitoral (rats only) (LX)
2 Zymbals Glands
2 Rectun (CX)
2 Mesenteric Lymph Node

1. Process for microscopic evaluation
2. Process for microscopic evaluation if grossly abnormal
CX = cross section
LX = longitudinal section

DETERMINATION OF MAXIMUM TOLERATED DOSE (MTD)

The MTD is defined as the dose which does not produce mortality or overt toxicity or no more than a 10% body weight decrement from control animals in a 90 day study.

PROC (procedure) PROBIT may be used from the SAS (Statistical Analysis System) to describe the proportion of animals exhibiting death or other toxic effects expressed in probits versus the dose. If an adequate range of proportions is obtained, which provides a satisfactory fit of the probit model, PROC PROBIT gives estimates of the doses, with confidence limits, which are predicted to produce toxic effects in 1%, 2%, etc. of the animals.

For 90 day animal body weights, a regression analysis may be used to predict average body weights with confidence limits as a function of dose. If the differences in body weights among doses are changing with time, it may be necessary to use the procedure described by Johnson (J. Tox. Envir. Hlth. 7: 307-316, 1981) to predict adult body weights.

MEMORANDUM

DEPARTMENT OF HEALTH AND HUMAN SERVICES
PUBLIC HEALTH SERVICE
FOOD AND DRUG ADMINISTRATION

TO: [redacted]

DATE: October 1, 1981

FROM: Principal Investigator, Experiment No. 376

SUBJECT: Animal Sacrifice Schedule, Experiment No. 376

Animals of Experiment No. 376 shall be removed from their feed and delivered to the Pathology Necropsy room in the PM prior to their scheduled sacrifice date as outlined on the attached animal sacrifice schedule. The animals will be fasted from the time of food removal until sacrifice but will be maintained on water ad libitum at all times.

Selected animals will be bled by oribital puncture and serum samples shall be prepared for SGOT and SGPT determinations. Serum samples shall be refrigerated until the analysis is performed, but not held more than four days. Because of the several hours required to collect and prepare the samples, the order of bleeding will be randomized among the different treatment groups and sexes sacrificed on a given day. Animals to be selected for these determinations are indicated on the attached sacrifice schedule by "cc" for clinical chemistry.

All animals shall be necropsied and tissues prepared for histopathological examination as outlined in the protocol. Histological examination shall begin with control animals and progress from the highest dose groups through decreasing dose groups until a "no effect" level is reached. The remaining dose levels shall be omitted from examination.

[redacted]

ANIMAL SACRIFICE SCHEDULE

Exp. No. 376 NTP No. 05010-02

Sacrifice Date*	Day	Room	Rack	Cage Numbers	Pathology Code*	Total Cages	Sex Dos
							Ra:
Oct. 6, 1981	Tuesday	151	01	5,6,7,8	cc	4	M/0
			04	41,42,43,44	cc	4	F/0
			02	17,18,19,20	cc	4	M/6
Oct. 7, 1981	Wednesday	151	03	29,30,31,32	cc	4	F/6
			01	9,10,11,12		4	M/2
			04	45,46,47,48		4	F/2
Oct. 8, 1981	Thursday	151	02	21,22,23,24	cc	4	M/1
			03	25,26,27,28	cc	4	F/1
			01	1,2,3,4		4	M/4
Oct. 9, 1981	Friday	151	04	37,38,39,40		4	F/4
			02	13,14,15,16	cc	4	M/1
			03	33,34,35,36	cc	4	F/1
							Mic
Oct. 13, 198	Tuesday	151	05	55,56,57	cc	3	M/0
			05	76,77,78	cc	3	F/0
			05	58,59,60	cc	3	M/1
Oct. 14, 198	Wednesday	151	05	73,74,75	cc	3	F/1
			05	52,53,54		3	M/7
			05	70,71,72		3	F/7

* Indicate in cover memo Date and Time of animal removal and any specific pathology requirements.

ANIMAL SACRIFICE SCHEDULE

Exp. No. 376 NTP No. 05010-02

Sacrifice Date*	Day	Room	Rack	Cage Numbers	Pathology Code*	Total Cages	
Oct. 15,1981	Thursday	151	05	64,65,66	cc	3	M/3
			05	79,80,81	cc	3	F/3
			05	49,50,51		3	M/1
Oct. 16,1981	Friday	151	05	67,68,69		3	F/1
			05	61,62,63	cc	3	M/8
			05	82,83,84	cc	3	F/8

* Indicate in cover memo Date and Time of animal removal and any specific pathology requirements.

Program Resources, Inc.

National Center for Toxicological Research • Jefferson, Arkansas 72079 • (501) 541-4554

DATE: July 21, 1981

TO: [redacted]

Approved [redacted] Date 8/5/81
Disapproved / Date
Principal Investigator:
[redacted]

Reviewed [redacted] Date 8-13-81
Quality A[redacted] Director
[redacted]

FROM: [redacted]

SUBJECT: ESS Recap for Experiment 376
90 day Subchronic-Doxylamine Study - NTP 5013-02

Experiment 376 is being conducted in room 151, building 5. A total of 288 animals of two species (1A-strain rats and 1H-strain mice) of males and females were automatically allocated. The rats were allocated Thursday July 02, 1981 and dosing started Monday July 06, 1981. The mice were allocated Wednesday July 08, 1981 and dosing started Wednesday July 15, 1981. Feed consumption will be recorded once a week for 13 weeks.

The dead or moribund animals will be removed with disposition Pathology (Histology). The animals living until the 90 day sacrifice will be removed as terminal with disposition Pathology (Histology).

ATTACHMENT A: RACK CONFIGURATION

ATTACHMENT B: TREATMENT TABLE DEFINITION

ATTACHMENT C: ANIMAL ROOM SCHEDULE

A T T A C H M E N T A

NOTE: THE INDIVIDUAL CAGE BLOCK INCLUDES START DATE

NATIONAL CENTER FOR TOXICOLOGICAL RESEARCH
RACK CONFIGURATION
EXPERIMENT: 376 ROOM: 151 RACK: 01

SIDE: A
STRAIN: 1A SEX: M

0001 CI 003 81187	0005 AW 001 81187	0009 DZ 005 81187			
0002 CI 003 81187	0006 AW 001 81187	0010 DZ 005 81187			
0003 CI 003 81187	0007 AW 001 81187	0011 DZ 005 81187			
0004 CI 003 81187	0008 AW 001 81187	0012 DZ 005 81187			

SIDE: B
STRAIN: 00 SEX:

NATIONAL CENTER FOR AICOLOGICAL RESEARCH
RACK CONFIGURATION
EXPERIMENT: 976 ROOM: 151 RACK: 03

SIDE: A						SIDE: B					
STRAIN: IA SEX: F						STRAIN: 00 SEX:					
0025	0029	0033									
CX	GF	GR									
004	006	002									
81187	81187	81187									
0026	0030	0034									
CX	GF	GR									
004	006	002									
81187	81187	81187									
0027	0031	0035									
CX	GF	GR									
004	006	002									
81187	81187	81187									
0028	0032	CC36									
CX	GF	GR									
004	006	002									
81187	81187	81187									

NATIONAL CENTER FOR TOXICOLOGICAL RESEARCH
RACK CONFIGURATION
EXPERIMENT: 376 ROOM: 151 RACK: 04

SIDE: A
STRAIN: 1A SEX: F

SIDE: B
STRAIN: 00 SEX:

0037 CI 003 81187	0041 AW 001 81187	0045 DZ 005 81187							
0038 CI 003 81187	0042 AW 001 81187	0046 DZ 005 81187							
0039 CI 003 81187	0043 AW 001 81187	0047 DZ 005 81187							
0040 CI 003 81187	0044 AW 001 81187	0048 DZ 005 81187							

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NATIONAL CENTER FOR T. .COLOGICAL RESEARCH
RACK CONFIGURATION
EXPERIMENT: 376 ROOM: 151 RACK: 05

SIDE: A
STRAIN: IN SEX: M

0049 GR 009 81196	0052 DQ 011 81196	0055 AW 007 81196	0058 DU 012 81196	0061 GG 008 81196	0064 DN 010 81196
0050 GR 009 81196	0053 DQ 011 81196	0056 AW 007 81196	0059 DU 012 81196	0062 GG 008 81196	0065 DN 010 81196
0051 GR 009 81196	0054 DQ 011 81196	0057 AW 007 81196	0060 DU 012 81196	0063 GG 008 81196	0066 DN 010 81196

SIDE: B
STRAIN: IN SEX: F

0067 GR 009 81196	0070 DQ 011 81196	0073 DU 012 81196	0076 AW 007 81196	0079 DN 010 81196	0082 GG 008 81196
0068 GR 009 81196	0071 DQ 011 81196	0074 DU 012 81196	0077 AW 007 81196	0080 DN 010 81196	0083 GG 008 81196
0069 GR 009 81196	0072 DQ 011 81196	0075 DU 012 81196	0078 AW 007 81196	0081 DN 010 81196	0084 GG 008 81196

ATTACHMENT B

TREATMENT TABLE
FOR
EXPERIMENT 376

TREATMENT NUMBER	DOSE CODE	COMPOUND	UNITS PPM	SEX/SPECIES	NO. OF CAGES	NO. OF ANIMALS/ CAGE-TOTAL CAGE	
001	AW	Control meal + Ethanol	0	M & F/Rats	8	3-24	
002	GR	Doxylamine	162	M & F/Rats	8	3-24	
003	CI	Doxylamine	405	M & F/Rats	8	3-24	
004	CK	Doxylamine	1012	M & F/Rats	8	3-24	
005	DZ	Doxylamine	2530	M & F/Rats	8	3-24	
006	FG	Doxylamine	6325.	M & F/Rats	8	3-24	
					48	144	TOTAL
007	AW	Control meal + Ethanol	0	M & F/Mice	6	4-24	
008	GG	Doxylamine	80	M & F/Mice	6	4-24	
009	GR	Doxylamine	162	M & F/Mice	6	4-24	
010	DN	Doxylamine	325	M & F/Mice	6	4-24	
011	DQ	Doxylamine	750	M & F/Mice	6	4-24	
012	DU	Doxylamine	1500	M & F/Mice	6	4-24	
					36	144	TOTAL
					84	288	GRAND TOTAL

ATTACHMENT C

ANIMAL REMOVALS:

Unscheduled - Dead and Moribund animals are to be removed as follows:

1. Remove as "DEAD" and "MORIBUND" utilizing the EIS terminal or the EIS manual form provided.
2. Fill out an EDCS "Single Animal Removal" form as well.

Scheduled - Remove as "terminal":

1. Use EIS terminal for the animal removal.
2. Assign CID's in the following order: N,L, and R.
3. Write earclip on back of CID cards and wrap them to cage card.
4. Take to Pathology.
5. No EDCS single animal removal form is needed.

CID CARDS:

The CID cards for this experiment have been issued in two blocks.

♂'s 1-144 have been designated for RATS and have RATS marked the cards.

♂'s 145-288 have been designated for MICE.

All animals are to be taken to Pathology.

DIAGNOSTIC ANIMALS:

None.

ENVIRONMENTAL MONITORING:

1. Environmental Test

Date	Day	Bedding & Waste 5 cages at random	Water All bottles	Air	Species	R
7-8-81	Wednesday			X		
7-9-81	Thursday	X	X		Rats	1-
8-5-81	Wednesday	X	X	X	Mice	5
9-2-81	Wednesday			X		
9-3-81	Thursday	X	X		Rats	1-
10-7-81	Wednesday	X	X	X	Mice	5

2. Chemistry Feed Samples

QA samples are to be collected from spares during the study when scheduled by the Chemistry Department.

DOSING OF ANIMALS:

See separate information.

[redacted]

Principal Investigator

June 29, 1981
Date

[redacted]

Program Resources, Inc.

June 29, 81
Date

LOCATION DIRECTORY

Location of Specimens, Data, and Supporting Documents:

The final repository for the original copy of the final report and microfiche copies of the protocol, its amendments and support documents, plus copies of animal husbandry logs, diet preparation records, and memoranda or other records related to the conduct of the experiment and preparation of the final report will be in the Quality Assurance Staff area, presently Building 15, Room 104. Analytical chemistry records and laboratory notebooks are stored in the Analytical Methods Branch area, currently Building 51, Rooms 113 and 122. Diagnostic records are stored in the Division of Microbiological Services, Building 60. Animal room temperatures and humidity records are in the Division of Facilities and Engineering and Maintenance storage area in the basement of Building 85. Pathology blocks and slides are stored in Building 5B, Room 130. Gross and microscopic opscan forms are stored within the Building 5 Computer Center adjacent to the data verification area. Computer storage of data is within the TDMS data base. Experimental files, including copies of reports requested in the protocol, are filed under the NTP or NCTR experiment number specified in the protocol and are located in the Office of Scientific Intelligence, Building 13. This is also the location of SOPs and direction for procedures used to support this and other similar NTP studies.

PRI Program Resources, Inc.
National Center for Toxicological Research
Jefferson, Arkansas 72079

DATE. January 7. 1982

TO: [redacted]
Principal Investigator

FROM: [redacted]

SUBJECT: Pathology Summary Report on Doxylamine and Tripelennamine

A. Doxylamine - Repeated Dose Study - Experiment 359

The study was terminated in mid April 1981. 36 each male and female rats and 36 each male and female mice were used in this study. Dose levels were from 0 to 2,000 ppm. All of the animals survived the entire study period. Treatment related lesions were identified on liver in both species. Rat livers exhibited various degrees of hepatic vacuolization where as the mouse livers exhibited small necrotic lesions. No other lesions related to the treatment were recognized.

Subchronic Study - Experiment 376

The study was terminated in mid October, 1981. 72 each male and female rats and 72 each male and female mice were used. Dose levels were from 0 to 6.325 ppm in rats and 0 to 1,500 ppm in mice.

All of the animals survived the entire study period. In rats, treatment related lesions were identified in livers and parotid salivary glands. Rat livers exhibited various severe degrees of hepatic vacuolization and/or fatty changes. The lesions were more severe in males than females.

Rat parotid salivary glands showed marked cytomegaly with basophilic and coarsely granulated or vacuolated cytoplasm. The glands were severely diffusely affected in the high doses but less severely affected in the lower dose.

In mice, treatment related lesions were identified only in liver as hepatic cytomegaly and karyomegaly. No obvious difference was noted between the males and females.

B. Tripelennamine - Repeated Dose Study - Experiment 369

The study was terminated in mid May, 1981. Dose levels were from 0 to 6,000 ppm in rats and 0 to 2,400 ppm in mice.

Rats - All 12 rats in the highest dose died between 7 to 13 days of treatment. Treatment related lesions were identified as thymic cortical atrophy and hepatocellular atrophy in the highest dose group and hepatic vacuolization in the lower dose groups.

Mice - All of the animals survived the entire study period. The only treatment related lesions were identified in the liver as small foci of hepatic necrosis.

Subchronic Study - Experiment 379

The study was terminated in November, 1981. Dose levels were from 0 to 2,400 ppm in rats and 0 to 4,800 ppm in mice.

2 rats of the high dose levels did not survive until the termination of the study.

Histopathologic examinations have not been completed for the study. However, treatment related lesions have been identified in the liver, parotid salivary glands and bronchial mucosa in the highest dose level group of rats, and liver and parotid salivary glands in the highest dose level group of mice. They are summarized as follows:

Rats - Livers exhibited moderate degree of hepatic vacuolization and/or fatty change. However, the liver atrophy nor thymic cortical as observed in 6,000 ppm dose level of repeated dose study were not observed in this study.

Parotid salivary glands exhibited mild to severe cytomegaly with basophilic and coarsely granulated or vacuolated cytoplasm as seen in Doxylamine Repeated Dose Study.

Bronchial epithelia exhibited severe degree of vacuolar degeneration of the mucosa.

Mice - The liver was affected by mild to moderate cytomegaly and karyomegaly in the highest dose.

The parotid salivary glands were affected by increasing incidence of individual cell necrosis which appeared to be more severe than the regular individual cell necrosis due to normal cell turnover in the glands of this strain of mice.

[redacted]

[redacted]
Pathology Services Project

[redacted]/ar

PATHOLOGY REPORT

90-DAY SUBCHRONIC TOXICITY STUDY - DOXYLAMINE

The following represents a tabulation of gross and microscopic findings from 144 Fischer 344 rats and 144 $B_6C_3F_1$ mice. In each species 72 males and 72 females were given various doses of Doxylamine in the feed for 90 days. Dose levels for each group of 12 males and 12 females were: control, 162, 405, 1012, 2530 and 6325 ppm in rats, and control, 80, 162, 325, 750 and 1500 ppm in mice.

Complete necropsies were performed on all animals. Microscopic examination was performed on control animals and those in the highest dose group in accordance with the Basic Ordering Agreement.

Tissues were fixed in Bouin's solution for 18-24 hours, processed in an Autotechnicon and embedded in paraffin. Sections were cut at 6 µm and stained with hematoxylin and eosin. An occasional tissue or organ is missing for technical reasons.

In the highest dose group, treatment-related lesions were identified in the liver and parotid salivary gland in rats and only the liver in mice. Hence the liver was examined microscopically in all animals of each species. The parotid salivary gland had treatment-related lesions in the second highest dose group (2,530 ppm) and no treatment-related lesions showed in the third highest dose group. The 2 lowest doses were not examined histologically. Some tissues not specified for examination were inadvertently submitted to the pathologist. These included lung, kidney, spleen, pancreas, preputial gland and mammary gland in the lower dose group. They also received histopathologic examination. Only those with positive findings were included in the tables.

Pathology Report - 90-day Subchronic Toxicity Study - Doxylamine

RESULTS

Gross lesions and day of sacrifice are tabulated in Table 1 for rats and Table 3 for mice. Microscopic lesions are presented in Table 2 for rats and Table 4 for mice.

RATS

All rats survived until the termination of the study. Microscopic lesions which appeared to be compound-related were:

1. Liver

   The liver lesions consisted of severe fatty change in the two highest dose groups. The fatty change in this study was characterized by many medium-sized vacuoles in the cytoplasm around the nucleus which severely distended the cytoplasm. The lesions occupied all except the periphery of the hepatic lobule. The nature of the lesions was suggestive of severe progressive change. The lower dose groups or milder fatty change and hepatic cytoplasmic vacuolization was characterized by slightly enlarged hepatocytes with either granular amphophilic or regular eosinophilic cytoplasm containing numerous small vacuoles dispersed throughout the cytoplasm. The hepatic lesions were more severe in males than in females. Only the two lowest dose groups in males and 3 lowest dose groups in females did not contain lesions.

2. Parotid Salivary Gland

Marked cytomegaly with basophilic and coarsely granular or vacuolated cytoplasm occurred in rat parotid salivary glands. The glands were severely affected diffusely in the high dose group but less severely affected diffusely in the lower dose groups.

MICE

Treatment-related lesions were identified in the liver. They varied in nature. The lesions consisted of hepatic cell cytomegaly or karyomegaly which were in varying degree from mild to severe. The lesions were more severe in the higher dose group and very subtle in the lower dose group. Only the females in the lowest dose group did not have treatment-related lesions. In contrast, the prevalence of a mild degree of hepatic cell necrosis was approximately constant irrespective of dose. In only three animals was it extensive, one of them in the highest dose group and two in lower dose groups.

Hepatic cell cytomegaly and/or karyomegaly and hepatic cell necrosis appeared to be dose-related. They were present in approximately equal prevalence and degree in both sexes except females in the lowest dose group were unaffected.

Pathology Report - 90-day Subchronic Toxicity Study - Doxylamine

SUMMARY

Lesions attributable to Doxylamine were found in the liver and parotid salivary glands in male and female rats. The lesions consisted of fatty change or hepatic cell vacuolization in the males of the 3 highest dose groups and in females of the 2 highest dose groups. Marked cytomegaly of the acinar cells of the parotid salivary gland was found in both sexes of the 2 highest dose groups.

Compound related lesions were also observed in mice. The lesions consisted of hepatic cell cytomegaly or karyomegaly and hepatic cell necrosis.

The microscopic change in salivary glands is unusual. In seeking an interpretation of it, Dr. Bernard Tandler, School of Dentistry, Case Western Reserve University, was consulted on June 28 and 29, 1982 (see attached summary of consultants comments).

From that consultation it is clear that normal rat parotids contain occasional small patches of acinar cells of increased size and contain basophilic, cytoplasmic granularity which was recorded as "cytomegaly, focal" in our control and low dose groups. However, interpretation of the diffuse, apparently treatment-related lesions in our high dose group is also presumptive since many factors such as chemical, time since eating, fixation, processing artifact, can be contributed to the morphological changes.

[redacted]
Pathologist
Pathology Services Project

LEGEND FOR TABLES

NGL = no gross lesions observed

X = organ present

0 = required organ not observed

- = organ not present

+ = lesions present but not graded

1 = minimal

2 = mild

2D = mild & diffuse

2F = mild and focal

3 = moderate

3D = moderate & diffuse

4 = severe

4D = severe & diffuse

TABLE 1

OBSERVATIONS AT NECROPSY OF F344 RATS IN 90-DAY SUBCHRONIC TOXICITY STUDY OF DOXYLAMINE

DOSAGE	SEX	IDENTITY OF ANIMAL	GROSS OBSERVATIONS	DAYS ON EXPERIMENT
0 ppm	M	1-12	NGL	92
102 ppm	M	109-120	NGL	95
405 ppm	M	73-74	NGL	94
		75	There was an 8mm reddish hard mass protruding above the capsular surface	94
		76-84	NGL	94
1,012 ppm	M	85-96	NGL	94
2,530 ppm	M	37	NGL	93
		38-48	The liver is yellow	93
6,325 ppm	M	13	NGL	92
		14-15	The liver is yellow	92
		16-20	NGL	92
		21	There was an 8mm soft reddish mass protruding above the capsular surface	92
		22-24	NGL	92
0 ppm	F	25-30	NGL	92
		31	There was a 12mm yellow mass in the peritoneum	92
162 ppm	F	121-132	NGL	95
405 ppm	F	133-141	NGL	95
		142	There was a 12mm grey subcutaneous mass at the left side of the neck area	95
		143-144		95
1,012	F	97-108	NGL	94
2,530	F	61-72	NGL	93
6,325	F	49-56	The liver was yellowish	93
		57	The liver was enlarged and yellowish	93
		58-60	The liver was yellow	93

TABLE 2

INDIVIDUAL HISTOPATHOLOGY FINDINGS - 90-DAY SUBCHRONIC TOXICITY STUDY - DOXYLAMINE

RATS

CONTROL - TREATMENT GROUP 1

ORGAN	MALES												FEMALES											
	1	2	3	4	5	6	7	8	9	10	11	12	25	26	27	28	29	30	31	32	33	34	35	36
LUNG	X	X	X	X	X	X	X	X	X	0	X	X	X	X	X	X	X	X	X	X	X	X	X	X
congestion																			+					
lymphocytic accumulation			+	+		+	+	+	+		+	+	+	+			+		+	+			+	
HEART	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
myocardial damage			+		+		+		+	+		+					+							
chronic inflammation					+				+			+												
KIDNEY	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
proteinaceous casts																								+
pigmentation																								+
fibrosis																								+
chronic inflammation																								+
tubular cyst																								+
focal nephropathy																								+
LIVER	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
cytoplasmic vacuolization										2														
congestion																						+		
SPLEEN	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
lymphoid hyperplasia												+												
erythropoiesis							+		+										+					
pigmentation			+																					
congestion																	+							
PANCREAS	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
atrophy, acinar cell																							1	
islet cell hyperplasia																							1	1
INTESTINE	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
granulomatous inflammation of Peyer's Patch								+		+														

Table 2 - continued

ORGAN	MALES 1	2	3	4	5	6	7	8	9	10	11	12	FEMALES 25	26	27	28	29	30	31	32	33	34	35	36
LYMPH NODES	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
congestion							+																	
PAROTID GLAND	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
cytomegaly					2F						2F								2F					
MARROW	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
hyperplasia																			1					
UTERUS	-	-	-	-	-	-	-	-	-	-	-	-	X	X	X	X	X	X	X	X	X	X	X	X
squamous metaplasia																							+	
PITUITARY	X	X	X	X	X	X	X	X	0	X	0	X	0	X	X	X	X	X	X	X	X	X	X	X
cyst														+										
BLADDER	X	X	X	X	X	X	X	0	0	X	X	X	X	X	X	X	X	X	X	X	0	X	X	X
refractile spherules							+																	
MESENTERY																			X					
fat necrosis	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-	-	-

TABLE 2

INDIVIDUAL HISTOPATHOLOGY FINDINGS - 90-DAY SUBCHRONIC TOXICITY STUDY - DOXYLAMINE

RATS

DOSE 162 ppm - TREATMENT GROUP 2

ORGAN	MALES												FEMALES											
	109	110	111	112	113	114	115	116	117	118	119	120	121	122	123	124	125	126	127	128	129	130	131	132
LIVER	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
chronic inflammation						1	1				1													
cytoplasmic vacuolization											2													

TABLE 2

INDIVIDUAL HISTOPATHOLOGY FINDINGS - 90-DAY SUBCHRONIC TOXICITY STUDY - DOXYLAMINE

RATS

DOSE 405 ppm - TREATMENT GROUP 3

ORGAN	MALES												FEMALES											
	73	74	75	76	77	78	79	80	81	82	83	84	133	134	135	136	137	138	139	140	141	142	143	144
LIVER	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
necrosis											1													
chronic inflammation			1												1									1
granulomatous inflammation																							2	
cytoplasmic vacuolization				2								2												
MAMMARY GLAND	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	X	-	-
adenocarcinoma																						+		

TABLE 2

INDIVIDUAL HISTOPATHOLOGY FINDINGS - 90-DAY SUBCHRONIC TOXICITY STUDY - DOXYLAMINE

RATS

DOSE 1,012 ppm - TREATMENT GROUP 4

ORGAN	MALES												FEMALES											
	85	86	87	88	89	90	91	92	93	94	95	96	97	98	99	100	101	102	103	104	105	106	107	108
LIVER	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
fatty change	2	2	2	2	2	2	2			2	2													
necrosis			1																					
inflammation, chronic		1	1							1														
granulomatous inflammation																	2	2						
cytoplasmic vacuolization								2	2			3												
PAROTID GLAND	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
cytomegaly							2F		2F											2F				

TABLE 2

INDIVIDUAL HISTOPATHOLOGY FINDINGS - 90-DAY SUBCHRONIC TOXICITY STUDY - DOXYLAMINE

RATS

DOSE 2,530 ppm - TREATMENT GROUP 5

	MALES												FEMALES											
ORGAN	37	38	39	40	41	42	43	44	45	46	47	48	61	62	63	64	65	66	67	68	69	70	71	72
LIVER	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
fatty change	4	4	4	4	4	4	4	4	4	4	4	4											2	
necrosis				1			1																	
chronic inflammation				1			1		1									2	2					
cytoplasmic vacuolization														3		3			3			3		3
PAROTID GLAND	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
cytomegaly	4D	3D	3D	3D		3D	2F	3D	2F		2F		2F		2F	2F	2F				2F	2F	2F	

TABLE 2

INDIVIDUAL HISTOPATHOLOGY FINDINGS - 90-DAY SUBCHRONIC TOXICITY STUDY - DOXYLAMINE

RATS

DOSE 6,325 ppm - TREATMENT GROUP 6

ORGAN	MALES												FEMALES											
	13	14	15	16	17	18	19	20	21	22	23	24	49	50	51	52	53	54	55	56	57	58	59	60
LUNG	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
lymphocytic accumulation			+			+										+						+		+
HEART	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
myocardial damage	+																							
chronic inflammation	+																							
KIDNEY	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
nephrosis	+																							
pigmentation	+																							
LIVER	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
fatty change	3	4	4	4	4	4	4	4	4	4	4	4	2	3	3	3	2	2	3	3	3	3	3	3
necrosis	1																							
SPLEEN	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
perifollicular hyperplasia					+		+																	
pigmentation										+	+	+												
congestion						+															+			
PANCREAS	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
atrophy													1									1		
STOMACH	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
edema																				+				
LYMPH NODES	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
pigmentation											+													

Table 2 - continued

ORGAN	MALES 13	14	15	16	17	18	19	20	21	22	23	24	FEMALES 49	50	51	52	53	54	55	56	57	58	59	60
PAROTID GLAND cytomegaly	X 4D	X 4D	X 4D	X 4D	X 4D	X 4D	X 4D	X 4D	X 4D	X 4D	X 4D	X 4D	X 4D	X 4D	X 4D	X 4D	X 4D	X 4D	X 4D	X 4D	X 4D	X 4D	X 4D	X 4D
MARROW hyperplasia	X	X	X	X	X	X	X	0	X	X	X	X	X	X	X	X	X	X 1	X	X	X	X	X	X
PITUITARY cyst	X	X +	0	X	X	X	X	X +	X	X	X	X	0	X	X	X	X	X	X	X	0	0	0	0

TABLE 3

OBSERVATIONS AT NECROPSY OF $B_6C_3F_1$ MICE IN 90-DAY SUBCHRONIC TOXICITY STUDY OF DOXYLAMINE

DOSAGE	SEX	IDENTITY OF ANIMAL	GROSS OBSERVATIONS	DAYS ON EXPERIMENT
0 ppm	M	145-156	NGL	90
80 ppm	M	253-264	NGL	93
162 ppm	M	217-228	NGL	92
325 ppm	M	229-240	NGL	92
750 ppm	M	181-192	NGL	91
1,500 ppm	M	157-168	NGL	90
0 ppm	F	169-180	NGL	90
80 ppm	F	227-288	NGL	93
162 ppm	F	265-276	NGL	93
325 ppm	F	241-252	NGL	92
750 ppm	F	193-204	NGL	91
1,500 ppm	F	205-216	NGL	91