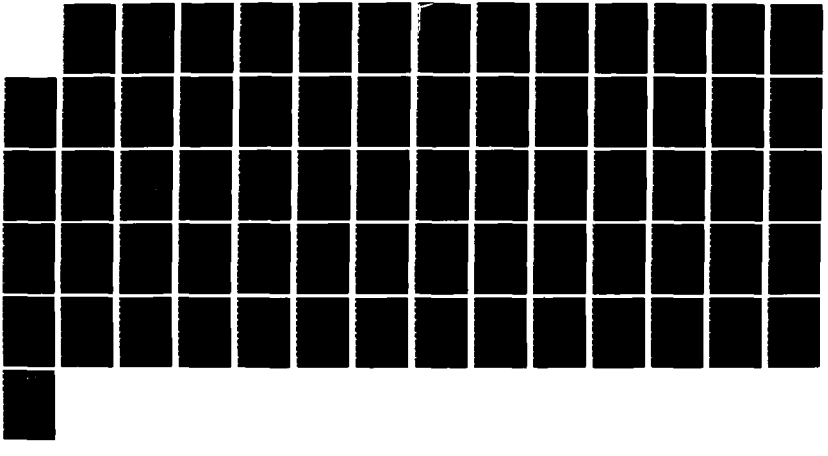
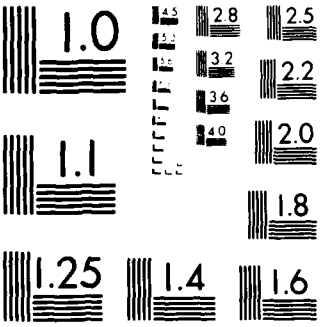
AD-A184 771 SUBCHRONIC TOXICITY STUDY ON 14-DITHIANE(U) NATIONAL CENTER FOR TOXICOLOGICAL RESEARCH JEFFERSON AR DIV OF COMPARATIVE TOXICOLOGICAL RESEARCH G J SCHIEFERSTEIN 1/1

UNCLASSIFIED 01 AUG 87 NCTR-E490 F/G 6/11 NL





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NCTR FINAL REPORT FOR EXPERIMENT 491

August, 1987

SUBCHRONIC TOXICITY STUDY OF 1,4-DITHIANE

ARMY PROJECT ORDER NUMBER 85PP5870

FDA IAG NUMBER 224-85-0013

Division of Comparative Toxicology
National Center for Toxicological Research
Jefferson, Arkansas 72079

NCTR FINAL REPORT FOR EXPERIMENT 491

August, 1987

SUBCHRONIC TOXICITY STUDY OF 1,4-DITHIANE

Prepared By: _____ Date: 12-11-86
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SUBCHRONIC TOXICITY STUDY ON 1,4-DITHIANE

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Division of Comparative Toxicology
National Center for Toxicological Research
Jefferson, Arkansas 72079

AUGUST 1987

Supported by

U. S. Army Medical Research and Development Command
Fort Detrick, Frederick, MD 21701-5012

Project Order 85 PP 5870

Project Officer: Gunda Reddy, Ph.D., D.A.B.T.
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UNCLASSIFIED

SECURITY CLASSIFICATION OF THIS PAGE

REPORT DOCUMENTATION PAGE

Form Approved OMB No 0704-0188 Exp Date Jun 30 1986

1a REPORT SECURITY CLASSIFICATION: UNCLASSIFIED	1b RESTRICTIVE MARKINGS: None
2a SECURITY CLASSIFICATION AUTHORITY	3 DISTRIBUTION/AVAILABILITY OF REPORT: APPROVED FOR PUBLIC RELEASE: DISTRIBUTION UNLIMITED.
2b DECLASSIFICATION/DOWNGRADING SCHEDULE	
4 PERFORMING ORGANIZATION REPORT NUMBER(S): E-490 and E-491	5 MONITORING ORGANIZATION REPORT NUMBER(S)
6a NAME OF PERFORMING ORGANIZATION: National Center for Toxicological Research; 6b OFFICE SYMBOL (If applicable): N/A	7a NAME OF MONITORING ORGANIZATION
6c ADDRESS (City, State, and ZIP Code): Jefferson, Arkansas 72079	7b ADDRESS (City, State, and ZIP Code)
8a NAME OF FUNDING/SPONSORING ORGANIZATION: Res. & Dev. Command; 8b OFFICE SYMBOL (If applicable):	9 PROCUREMENT INSTRUMENT IDENTIFICATION NUMBER: 85PP5870
8c ADDRESS (City, State, and ZIP Code): Frederick, MD 21701-5012	10 SOURCE OF FUNDING NUMBERS: PROGRAM ELEMENT NO.; PROJECT NO; TASK NO; WORK UNIT ACCESSION NO

11. TITLE (Include Security Classification)

Subchronic Toxicity Study on 1,4-Dithiane.

12. PERSONAL AUTHOR(S)

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13a TYPE OF REPORT	13b TIME COVERED	14 DATE OF REPORT (Year, Month, Day)	15 PAGE COUNT
Final Report	FROM 9/25/85 TO 8/1/87	1987 August 1	60

16. SUPPLEMENTARY NOTATION

17. COSATI CODES		
FIELD	GROUP	SUB-GROUP

18. SUBJECT TERMS (Continue on reverse if necessary and identify by block number)

Subchronic toxicity. 1,4-Dithiane (CAS Registry Number: 505-29-3). Rat. Anisotropic crystals. Eosinophilic granules.

19. ABSTRACT (Continue on reverse if necessary and identify by block number)

In a preliminary 14-day range-finding study, CD rats were gavaged daily at 0, 25, 50, 100, 210 and 420 mg/kg/day (6 rats/sex/dose). No overt toxicity or mortality was observed. There was a non-significant ($p>.05$) decrease in body weight at the 420 mg/kg/day dose in the males and females of 5 and 8 respectively.

In the 90-day subchronic study, CD rats were gavaged daily at 0, 105, 210, and 420 mg/kg/day (30 rats/sex/dose). No overt toxicity, treatment-related mortality or treatment-related clinical chemical, hematologic or ophthalmologic change was found. The female livers and the male kidneys were significantly ($p<.05$) heavier in the treated animals. There was deposition of anisotropic crystals of an undetermined chemical composition in the nasal olfactory mucosa of both sexes of the high and intermediate dose groups. Their presence in the low-dose group was characterized by a greater involvement in the females. The crystals were not observed in the control animals. Other treatment-related lesions were eosinophilic cytoplasmic granulations of the convoluted renal tubule cells of the high dose males. The

20 DISTRIBUTION/AVAILABILITY OF ABSTRACT: ☒ UNCLASSIFIED/UNLIMITED ☐ SAME AS RPT ☐ DTIC USERS	21 ABSTRACT SECURITY CLASSIFICATION: Unclassified
22a NAME OF RESPONSIBLE INDIVIDUAL: Judy Pawlus	22b TELEPHONE (Include Area Code): 301-663-7325; 22c OFFICE SYMBOL: SGRD-RMI-S

DD FORM 1473, 84 MAR 83 APR edition may be used until exhausted. All other editions are obsolete.

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NORM

Block 19. livers of the high dose females contained two hepatocellular lesions: minimal hypertrophy in the centrilobular region and minimal cytoplasmic vacuolation in the periportal region.

FOREWORD

Opinions, interpretations, conclusions and recommendations are those of the author and are not necessarily endorsed by the U.S. Army.

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____In conducting research using animals, the investigator(s) adhered to the "Guide for the Care and Use of Laboratory Animals," prepared by the Committee on Care and Use of Laboratory Animals of the Institute of Laboratory Animal Resources, National Research Council (NIH Publication No. 86-23, Revised 1985).

____For the protection of human subjects, the investigator(s) have adhered to policies of applicable Federal Law 45CFR56.

George J. Schieferstein 7-14-87

George J. Schieferstein, Ph.D. Date

EXECUTIVE SUMMARY

There is a paucity of toxicologic and pathologic information on 1,4-dithiane. This 90-day investigation was undertaken to determine the subchronic toxicologic and pathologic effect(s), if any, of 1,4-dithiane in the rat. Several doses (three doses per sex as well as a control) were used in order to obtain as much information as possible at non-toxic dose levels. For each dose, 30 animals of each sex were used in order to provide a sample size sufficient to detect any low-incidence pathologies. A complete pathology work-up was done in order to obtain as much pathology information as possible. A preliminary 14-day range-finding study was conducted in order to select a maximum tolerated dose for the 90-day study. Five dose levels (6 animals/sex/dose) and a control group (6 animals/sex) were used in the range-finding study in order to obtain toxicologic information over a wide range of doses. The results of the 14- and 90-day studies are summarized in this Final Report.

In a preliminary 14-day range-finding study, rats were gavaged daily at 0, 25, 50, 100, 210 and 420 mg/kg/day (6 rats/sex/dose). No overt toxicity or mortality was observed. There was a non-significant ($p > .05$) decrease in body weight at 420 mg/kg/day in the males and females of 5 and 8%, respectively. In the 90-day subchronic study, rats were gavaged daily at 0, 105, 210 and 420 mg/kg/day (30 rats/sex/dose). No overt toxicity, treatment-related mortality or treatment-related clinical chemical, hematologic or ophthalmologic change was found. The female livers and the male kidneys were significantly ($p < .05$) heavier in the treated animals. There was deposition of anisotropic crystals of an undetermined chemical composition in the nasal olfactory mucosa of both sexes of the high and intermediate dose groups. Their presence in the low-dose group was characterized by a greater involvement in the females. The crystals were not observed in the control animals. Other treatment-related lesions were eosinophilic cytoplasmic granulation of the convoluted renal tubule cells of the high dose males. The livers of the high dose females contained two hepatocellular lesions: minimal hypertrophy in the centrilobular region and minimal cytoplasmic vacuolation in the periportal region.

SUBCHRONIC TOXICITY STUDY OF 1,4-DITHIANE

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I. SPECIFIC AIMS

This 90-day study on 1,4-dithiane in the rat was undertaken in order to expand scientific knowledge of the compound's subchronic toxicologic and pathologic effects. 1,4-Dithiane is an environmental contaminant found in the vicinity of former mustard gas spills.

II. BACKGROUND

1,4-Dithiane has been identified as a ground water contaminant at certain military installations (Dr. Rosenblatt, personal communication, 1986). Since a computer-assisted literature search yielded little information on the compound's toxicity to man, experimental animals, or to other organisms, USATHMA (U. S. Army Toxic and Hazardous Materials Agency) requested USABRDL (U.S. Army Biomedical Research and Development Laboratory) to provide toxicologic information on 1,4-dithiane. Ohmoni et al. (1985) reported that 1,4-dithiane did not augment the antibody response to sheep erythrocytes in BALB/c mice.

In an Army-sponsored mutagenicity study on 1,4-dithiane conducted at Letterman Army Institute of Research, tester strains TA 98, TA 100, TA 135, TA 1537, and TA 1538 were exposed to doses of 1,4-dithiane ranging from 5 mg/plate to 0.00016 mg/plate. The 1,4-dithiane was not mutagenic under the experimental conditions used (Sano and Korte, 1985). An LD_{50} study on 1,4-dithiane in the Fischer 344 rat at American Biogenic Corporation showed the oral LD_{50} to be 3,680, 2,768 and 3,473 mg/kg, respectively, for the males, females and both sexes combined (Mayhew and Muni, 1986). In response to a requirement for the toxicologic and pathologic data, USABRDL instituted an Interagency Agreement between the FDA (for NCTR) and USABRDL for the 14-day study (NCTR Exp. 490) and for the subsequent 90-day study (NCTR Exp. 491) on September 25, 1985. The 14- and the 90-day studies began on January 21 and April 8, 1986, respectively. The preliminary results of this project were presented to and discussed with USABRDL at its annual research review on Monday, September 15, 1986. The results were presented at the Society of Toxicology Meeting in Washington, DC in February, 1987. The abstract (Schieferstein et al, 1987) for the February, 1987 meeting was reviewed by and discussed with USABRDL (See Appendix 1 for a copy).

III. EXPERIMENTAL METHODS AND MATERIALS

A. Dose Preparation

The 1,4-dithiane (CAS Registry Number: 505-29-3) was dissolved in sesame oil and administered by gavage in both the 14- and 90-day studies. Each batch was characterized before use and after dosing by the Division of Chemistry, NCTR, by GC, GC/MS and NMR. The Division of Chemistry performed a 90-day stability study in sesame oil on 1,4-dithiane at two concentrations (2 and 35 mg/ml). The purity determination and other gas chromatographic chemical analyses were performed using a Varian Model 3700 Gas Chromatograph equipped with a flame ionization detector and a 3' x 2 mm, i.d, glass column packed with 3% SP-2250 on Supelcoport 100/120 mesh. The column oven temperature was set at 80°C and the injection port and detector were operated at 120°C and 280°C, respectively. The carrier gas nitrogen flowed at a rate of 30 mL/minute. All injections were 2 uL using a 10uL syringe (#701, Hamilton Co., Reno, Nev.) fitted with a Chaney adapter. The solutions were prepared at 1

mg mL in methylene chloride (J. T. Baker) for direct comparison to a 1,4-dithiane (Aldrich Chemical Co.) reference standard. Gas chromatographic mass spectral (GC/MS) analyses of the 1,4-dithiane samples were performed on a Finnigan Model 4023 quadrupole mass spectrometer (FINNIGAN-MAT, San Jose, CA) operated in the standard electron impact (EI) mode with a source temperature of 270°C. Separation was achieved on a 30 meter DB5-30N bonded phase capillary column (J & W SCIENTIFIC, Rancho Cordova, CA) operated at 20 psig inlet head pressure. Column oven temperature was controlled at 60°C for 2 minutes and then ramped to 240°C at 15°C/min. Injections were made on a Grob injector operated in the splitless mode with a septum sweep rate of 30 mL/min and a delay of 35 sec. Retention time for the major component was 4.55 min. Mass spectra were visually inspected and computer matched to the NIH/EPA 31,000 spectral library. Because the samples were dissolved in methylene chloride, only one component was observed. Library matches were on the order of 45 because the mass spectrometer was not scanned below 50 daltons due to intense ions from the solvent. The proton Nuclear Magnetic Resonance (NMR) analyses were performed on a Bruker WM-500 instrument (Bruker Instruments, Inc., Manning Park, Billerica, MA) at room temperature (300°K) in chloroform-d_1 (Merck Isotopes, Ranway, NJ). One mg of recrystallized 1,4-dithiane (see V. RESULTS A. Chemical Analyses for a discussion of the need for recrystallization) was dissolved in chloroform-d_1. One transient was collected for each sample with a 75 degree flip angle (11.5 u second 90 degree flip), 32K data points, 7024 Hz window, and 20 sec delay prior to pulse. The data (FID) was treated with a 0.5 Hz line broadening prior to Fourier transformation. The spectra were analyzed for methylene chloride (singlet, approximately 5.3 ppm) and ethanol (methylene quartet at 3.55 ppm and methyl triplet at 1.11 ppm) by chemical shift using residual protiated proton chloroform (7.24 ppm) as an external reference standard. The quantitation of the observed impurities were accomplished by integration of impurity resonances with respect to the 1,4-dithiane resonance.

There is only one manufacturer of 1,4-dithiane in the U.S.: Fairfield Chemical Co., (P.O. Box 20, Blythewood, SC 29016). Aldrich Chemical Co., Inc. (940 W. St. Paul Ave., Milwaukee, WS 53233) resells 1,4-dithiane that it has purchased from Fairfield Chemical Co. After the 14-day study had been completed but before the start of the 90-day study, the 1,4-dithiane was found to be contaminated with a small percentage (0.2%) of methylene chloride. All 1,4-dithiane used in the 90-day study was free of methylene chloride, a known animal carcinogen. The methylene chloride contaminant was removed by recrystallizing the contaminated 1,4-dithiane from absolute ethanol. The recrystallized 1,4-dithiane contained 1.5 $\pm$ 0.5% ethanol. The following lots of 1,4-dithiane were received at NCTR.

1. Fairfield Chemical Co. lot 102 (100 gm). This methylene-chloride-contaminated lot was used in the 14-day range-finding study and in the chemical stability study.

2. Fairfield Chemical Co. lot 102A (200 gm). This lot contained methylene chloride that was removed by recrystallization from ethanol (recrystallization #2). After recrystallization this lot was used for dosing in the 90-day toxicity study.

3. Fairfield Chemical Co. lot 103 (700 gm). Initially 300 gm of this methylene-chloride-contaminated lot was recrystallized (recrystallization #1). Subsequently, the other 400 gm was recrystallized at the same time lot 102A was recrystallized (recrystallization #2). After recrystallization lot 103, lot 103A, and lot 102A were used for dosing in the 90-day toxicity study.

4. Fairfield Chemical Co. lot 103A (700 gm) contained methylene chloride and was recrystallized (recrystallization #2).

5. Aldrich Chemical Co. lot 03222 CM (200 gm). This lot was found to be free of methylene chloride and, accordingly, was used for dosing in the 90-day toxicity study without recrystallization.

6. Fairfield Chemical Co. lot 106 (800 gm). Four hundred grams of this methylene-chloride-contaminated lot was recrystallized (recrystallization #3) and was used for dosing in the 90-day toxicity study. Four hundred grams of methylene-chloride-contaminated material remains stored at NCTR as does about 100 gm of recrystallization #3.

The results of the 90-day chemical stability study are given in Table 1. Within the limits of experimental variation, there was no chemical deterioration of the 2 mg/ml or 35 mg/ml solutions of 1,4-dithiane during the 90-day study period. In the 14-day study 2.0, 8.75 and 35 mg/ml solutions of 1,4-dithiane in sesame oil were used. In the 90-day study, only a 35 mg/ml solution was used. The dosing solutions were stored for a maximum of 3 weeks in a freezer.

Table 1. Results of the 90-day chemical stability study on a 2 mg/ml and a 35 mg/ml solution of 1,4-dithiane in sesame oil.

	Solution (mg/ml)	
Day	2	35
0	1.77 ± 0.01[a](-)[b]	32.73 ± 0.94[a](-)[b]
1	1.68 ± 0.04 (94.9)	33.43 ± 1.36 (102)
2	1.64 ± 0.04 (92.6)	33.00 ± 1.23 (101)
7	1.62 ± 0.03 (91.5)	33.63 ± 0.40 (103)
14	1.63 ± 0.10 (92.1)	33.04 ± 0.59 (101)
21	1.64 ± 0.04 (92.6)	33.77 ± 0.58 (103)
28	1.67 ± 0.03 (94.4)	32.70 ± 0.77 (100)
42	1.59 ± 0.08 (89.8)	34.34 ± 1.01 (105)
56	1.77 ± 0.07 (100)	32.98 ± 0.75 (101)
90	1.81 ± 0.06 (102)	34.45 ± 0.86 (105)

[a] Mean ± SD of 3 determinations.
[b] Percent of day 0 value.

The solutions were thawed on the day of use. Any unused dosing solution was incinerated.

The various lots of 1,4-dithiane were checked by flame ionization gas chromatography, by GC-MS, and by NMR. The lots were identified by GC-MS and by NMR as 1,4-dithiane before being used as dosing solutions. The 1,4-dithiane remaining in lot 106 (recrystallization #3) was certified to be 1,4-dithiane by GC-MS and by NMR after the end of dosing. The percent purity by flame ionization gas chromatography of the various lots against a reference standard (Aldrich Chemical Co. lot 3210 KL) was:

Lot	% Purity
Fairfield lot 102	97.4
Fairfield lot 102A	98.2
Fairfield lot 103	>99.9
Fairfield lot 103A	>98.4
Aldrich lot 03222 CM	>99.9
Fairfield lot 106	100.5

The various lots were 97.4% or greater pure 1,4-dithiane. The dosing solutions concentration in the 90-day toxicity study was adjusted for the percent purity of a given lot.

The results of the bedding and feed analyses are shown in Tables 2 and 3, respectively. All results are within NCTR's Guidelines for acceptable bedding and feed.

Table 2. Results of the analyses on the bedding.

	NCTR Lot Numbers					NCTR Guidelines	
Variable analyzed	2	3	4	5	6	Minimum	Maximum
Moisture (%)	6.2	2.4	5.0	4.8	4.1		8
Sizing, 20 (% retained)	92.9	84.6	94.8	91.9	88.7		
Sizing, 08 (% retained)	6.2	14.3	4.1	6.8	10.6		17
Sizing (% passed into pan)	0.98	1.1	1.1	1.3	0.72		6
Pentachlorophenol (ppm)	<MDL[a]	<MDL	<MDL	<MDL	<MDL		10,000
Polychlorinated biphenyls (ppb)	<MDL	<MDL	<MDL	<MDL	<MDL		2

[a] Minimum detectable level.

Table 3. Results of chemical analyses on the feed. Lot 85121511 was used in the 14-day study. Lot 85121021 was used in the 90-day study.

	Lot Number		NCTR Guidelines	
Variable analyzed	85121511	85121021	Minimum	Maximum
Arsenic (ppb)	530	520		1000
Total fat (%)	7.1	5.4	4.2	
Vitamin B_1 (mg/g)	0.09	0.078	0.075	0.125
Selenium (ppm)	0.26	0.33	0.05	0.65
Total protein (%)	21.0	20.1	18	
Mercury (ppm)	<0.05	<0.05		0.1
Lead (ppm)	1.27	0.57		1.5
Cadmium (ppb)	106	180		250

B. Dose Certification

The Chemistry Division analyzed the concentrations of each 1,4-dithiane stock solution (35 mg/ml) used in the 14- and 90-day studies. In the 14-day study, the animals were gavaged with appropriate volumes of either a 8.75 or a 2 mg/ml solution that were made by dilution of the 35 mg/ml solution. In the 90-day study only the 35 mg/ml solution was used. The acceptance criterion in the 90-day study was ± 10% of the target concentration. There was no acceptance criterion in the 14-day study. The dosing solutions, which had been kept frozen, were thawed and sonicated on the day of their use. The dosing solutions were kept at ambient temperature in the dosing room until use. Any leftover solutions were placed in a plastic bag and disposed of by incineration.

The flame ionization gas chromatographic determinations of the dosing solutions used in the 14- and 90-day studies is given in Table 4. In the 90-day study there was a ± 10% of target concentration acceptance criterion. All the dosing solutions used in the 90-day study were within ± 5% of the target concentration of 35 mg/ml (Table 4).

C. Dosing Procedure

In the 14-day study, CD rats obtained from the NCTR breeding colony were gavaged daily for 14 consecutive days. The rats were randomly allocated to the various dose levels at four weeks of age (acceptable age range : 24-33 days of age) and were started on dose at 5 weeks of age (acceptable age range 31-40 days of age.) The animals were randomly assigned to the various treatment levels on a weight basis. The animals were weighed and arranged in order from the lightest to the heaviest. The lightest and/or heaviest animals from the allocation pool of animals were discarded until 36 animals/sex remained. The allocation procedure that was used assured that six of the lighter animals were randomly assigned (one/dose) to each of the six dose levels. Then six of the successively next heavier animals were assigned until all the animals had been assigned. Accordingly each dose level had six animals ranging from light to heavy. The rack configurations for both sexes are given in Table 5.

Table 4. Concentrations of the dosing solutions used in the 14- and 90- day toxicity studies. Re: recrystallization

Study	Date	Volume (l)	Lot Number	Target concentration (mg/ml)	Observed Concentration (mg/ml)	Percent of Target Concentration
14-day	1/17/86	0.2	102	2.00	1.73 ± 0.01[a]	86.5
	1/17/86	0.5	102	8.75	7.91 ± 0.06	90.4
	1/17/86	1	102	35.00	35.9 ± 0.6	102
90-day	4/7/86	2	103, Re 1	35.0	33.4 ± 0.2[b]	95.4
	4/17/86	4	103, Re #1	35.0	35.1 ± 0.5	100.0
	5/5/86	4	102,103, 103A, Re#2	35.0	35.1 ± 0.5	100.0
	5/13/86	4	Re #2	35.0	34.7 ± 0.3	99.1
	5/21/86	4	Re #2	35.0	33.9 ± 0.2	96.8
	5/30/86	4	Re #2	35.0	34.2 ± 0.4	97.7
	6/9/86	4	Mixture of Re #1 and Re #2	35.0	34.8 ± 0.5	99.4
	6/19/86	4	03222 CM	35.0	35.0 ± 0.6	100.0
	6/26/86	2	03222 CM, Re #1, and Re #2 Mixture	35.0	34.6 ± 0.1	98.8
	7/1/86	2	106, Re #3	35.0	34.7 ± 1.0	99.1
	7/2/86	3	Re #3	35.0	34.6 ± 0.9	98.8

[a] Mean ± SD of 3 determinations on one aliquot.
[b] Mean ± SD of 5 determinations on one aliquot.

Table 5. Rack configurations in the 14-day study.

Males

Rack 1			Rack 2		
[a]					
1[b] - 210[c]	4 - 0	7 - 100	10 - 25	13 - 420	16 - 50
2 - 210	5 - 0	8 - 100	11 - 25	14 - 420	17 - 50
3 - 210	6 - 0	9 - 100	12 - 25	15 - 420	18 - 50
[a]					

Females

Rack 3			Rack 4		
[a]					
19[b] - 210[c]	22 - 0	25 - 100	28 - 25	31 - 420	34 - 50
20 - 210	23 - 0	26 - 100	29 - 25	32 - 420	35 - 50
21 - 210	24 - 0	27 - 100	30 - 25	33 - 420	36 - 50
[a]					

[a] The top and bottom shelf levels were not used.
[b] Cage number.
[c] Dose (in mg/kg/day).

There were two rats per cage. The animals were weighed on day 1 (when dosing began), on day 7, and on day 14 (when dosing stopped). The dose for days 1 to 7 was determined by the day 1 weight. The dose for days 8 to 14 was determined by the day 7 weight. All survivors were euthanized on day 15. Food (NIH-31) and water (filtered potable NCTR) consumption was determined weekly. The animal weight and observations, food and water consumption were recorded on manual forms.

In the 90-day study, CD rats obtained from the NCTR breeding colony were gavaged daily for 90 consecutive days. The allocation, dosing ages, housing, and observations were performed in the same manner as in the 14-day study. The rack configuration for both sexes are given in Table 6. Each cage contained a no-ear and a left-ear clipped animal. Males were started on dose on a Tuesday, (April 8, 1986) and the females were started on dose on the following Thursday (April 10, 1986). The males were sacrificed 13 weeks later on a Monday through Wednesday (89 to 91 days on dose) and the females were sacrificed 13 weeks later on a Wednesday through Friday (89 to 91 days on dose). The animals were not gavaged on the day of terminal sacrifice. Food (NIH-31) and water (filtered potable NCTR) consumption were determined on a weekly basis. Dosing on day 1 (when the study started) to day 7 was determined by the day 1 animal weight. Each succeeding week's dose was determined in a similar manner. There were 10 gm increments in the dosing chart and the gavage volume varied with the animals' weight. The cages were rotated one level down on the rack every two weeks on dose. The cages on the bottom shelf were placed on the top shelf when cage rotation occurred. The control and high-dose males were gavaged twice per day from week 8 to week 13 on dose in the 90-day study in order to keep the volume per gavaging less than 5 ml.

Table 6. Rack configuration in the 90-day study.

Males				Females			
Rack 1				Rack 4			
1[a]-420[b]	6- 105	11-0	16-210	61-0[b]	66-210	71-420	76-105
2- 420	7-105	12-0	17-210	62-0	67-210	72-420	77-105
3- 420	8-105	13-0	18-210	63-0	68-210	73-420	78-105
4- 420	9-105	14-0	19-210	64-0	69-210	74-420	79-105
5- 420	10-105	15-0	20-210	65-0	70-210	76-420	80-105
Rack 2				Rack 5			
21 -0	26 -210	31 -105	36 -420	81 -420	86 -105	91 -210	96 -0
22[c]-0	27[c]-210	32[c]-105	37c-420	82[c]-420	87[c]-105	92[c]-210	97[c]-0
23[c]-0	28[c]-210	33[c]-105	38[c]-420	83[c]-420	88[c]-105	93[c]-210	98[c]-0
24[c]-0	29c-210	34[c]-105	39[c]-420	84[c]-420	89[c]-105	94[c]-210	99[c]-0
25 -0	30 -210	35 -105	40 -420	85 -420	90 -105	95 -210	100 -0
Rack 3				Rack 6			
41-105	46-420	51-210	56-0	101-210	106-0	111-105	116-420
42-105	47-420	52-210	57-0	102-210	107-0	112-105	117-420
43-105	48-420	53-210	58-0	103-210	108-0	113-105	118-420
44-105	49-420	54-210	59-0	104-210	109-0	114-105	119-420
45-105	50-420	55-210	60-0	105-210	110-0	115-105	120-420

[a] Cage number.
[b] Dose (in mg/kg/day).
[c] Animals used for clinical chemistry.

D. Experimental Design

The experimental design for each sex in the 14-day study was:

Dose (mg/kg body weight/day)	Number of animals
0	6
25	6
50	6
100	6
210	6
420	6
Total per sex	36
Total on test	72

In both the 14-day and 90-day studies the control animals' weights were averaged and each control animal was dosed with the same volume of sesame oil as received by the high dose group. The 14-day study was conducted from January 21 to February 3, 1986. Survivors were euthanized on February 4,

1986. The 90-day study started on dose on April 18 and had its final sacrifice on July 18, 1986.

The experimental design for each sex in the subsequent 90-day study was:

Dose (mg/kg body weight/day)	Number of animals
0	30
105	30
210	30
420	30
Total per sex	120
Total on test	240

The Army Project Officer approved the high dose suggested by the Principal Investigator for use in the 90-day study. As in the 14-day study, the control animals' weights were averaged and each control animal was dosed with the volume of sesame oil it would have received if it had been on the high dose in order to detect any vehicle-induced effects. The animals were dosed and housed in Building 4 at NCTR from April 8 to July 11, 1986.

E. Pathology

No pathological evaluations were done in the 14-day study. In the 90-day study:

1. A complete necropsy was performed on all rats. Dead and moribund animals removed before the terminal sacrifice had a complete necropsy but histopathologic examination of these animals was limited to the nose because of the anisotropic crystals found in the olfactory mucosae of the 90-day sacrificed animals. Tissues from terminal sacrifice animals were processed for histopathologic examination. At the terminal sacrifice the liver, thymus, spleen and brain were weighed.
2. An ophthalmologic examination was performed by a board-certified veterinary ophthalmologist (Robert J. Munger, DVM of Dallas, TX) on all animals before the start of dosing (on April 7, 1986) and during the twelfth week of dosing (on June 30, 1986).

3. A detailed histopathology evaluation was performed on all high-dose and control animals in the terminal sacrifice. An "inverse-pyramid" procedure was used. Organs having treatment-related lesions in the high-dose group were examined at successively lower doses until a "no-effect" dose was found or until all animals were examined.

4. Analysis of serum aspartate aminotransferase (AST), alanine aminotransferase (AAT), serum sorbitol dehydrogenase (SDH), amylase and lactic dehydrogenase isoenzyme and a complete blood count (CBC) with leukocyte differential and reticulocyte count were performed on 6 animals/sex in the three dose groups and in the control group on the day before dosing began and at 30, 60, and 90 days on dose. Samples for clinical chemistry were taken in sterile, silicone-coated tubes. Samples for hematology used EDTA as the anticoagulant. The samples were collected within a 3-hour period and assays were completed on the day of collection.

F. Statistical Methods

An analysis of variance was performed on the body weight data in the 14-day range-finding study.

In the 90-day study, PROC CHRONIC (developed at NCTR) was used to statistically test for dose-response trends in the olfactory nasal crystal data. An analysis of variance was run on the body weight data. The analysis of the food and water consumption data was performed using the repeated measurements (profile) technique described in Multivariate Statistical Methods. When a significant dose effect was found, a Bonferroni multiple comparison between each dose and the control was calculated. The organ weight and the organ over terminal body weight data were analyzed by a linear regression analysis (SAS Version 5.08, Proc. Reg.). The clinical chemistry and hematology data were also evaluated by a linear regression analysis (SAS Version 5.08, Proc. Reg.). The clinical chemistry and hematologic values before the start of dosing were subtracted from the values obtained at 30, 60 and 90 days on dose and the resultant differences were statistically analyzed by a linear regression analysis. The ratios of the values at 30, 60, and 90 days on dose to the pre-dosing values were also analyzed. The values at 30, 60, and 90 days on dose were pooled and the differences from pre-dosing values were analyzed. Finally, the pooled data were analyzed as fractions of the pre-dosing values. Since there was no days-on-dose effect, the pooling increased the power of the statistical tests.

IV. RESULTS

A. 14-Day Range-finding Study

The rats were gavaged daily from January 21 to February 3, 1986 and were euthanized on February 4, 1986. There was no mortality or overt toxicity. The water consumption, food consumption and weight gain data are given in Table 7. There is no apparent treatment-related effect upon water - food consumption. In the 14-day study in the males and females there w non-significant ($p>.05$) 5.3 and non-significant ($p>.05$) 8% weight los respectively, at the high dose (420 mg/kg/day). In view of the absence toxicity at the high dose level, and in view of the low solubility (35 mg/m' of the 1,4-dithiane in sesame oil, 420 mg/kg/day was chosen for the high dose in the 90-day study.

B. 90-Day Subchronic Study.

1. Body weight, food consumption, and water consumption. The body weight, food consumption, and water consumption data are presented in Tables 8 to 13 and in Figures 1, 2, and 3, respectively. The left-ear clipped control male in cage 14 had malocclusion (a bite defect) and was excluded from all statistical analyses. An ANOVA on the body weight data showed no significant ($p>.05$) treatment-related effects.

In the statistical analyses of food consumption data in the females, the parallelism hypothesis was rejected. This mandated looking at each week separately rather than the mean of all weeks when comparing dose levels to determine if any significant differences existed. A separate univariate ANOVA for each week was run to determine if a dose effect existed. The p values for the tests for dose effect were:

Table 7. Effect of 1,4-dithiane upon water consumption, food consumption, and weight gain in the 14-day range-finding study.

Sex	Dose mg/kg/day	Water Consumption (g/cage/wk) for: Week 1		Water Consumption (g/cage/wk) for: Week 2		Food Consumption (g/cage/wk) for: Week 1		Food Consumption (g/cage/wk) for: Week 2		Weight Gain (g) for: Week 1[d]		Weight Gain (g) for: Week 2[e]		Weight Gain (g) for: Week 1 & Week 2[f]	
M	0	463.2[a]	---	550.2[a]	---	250.7[a]	---	295.5[a]	---	49.5[b]	---	51.3[b]	---	100.7[b]±4.4[g]	---
	25	446.6	96.3[c]	560.3	102[c]	299.7	115[c]	340.8	115[c]	54.0	109[c]	53.2	104[c]	107.2±5.2	106.0[c]
	50	471.0	102	499.2	91	291.2	112	319.6	108	51.3	104	38.8	75.8	90.1±2.9	89.5
	100	456.2	98.5	513.2	93	294.6	113	329.8	112	51.5	104	47.3	92.4	98.7±4.6	98.1
	210	475.6	96.2	573.6	104	292.9	112	344.8	117	49.1	99.1	49.8	97.3	98.9±4.5	98.2
	420	487.4	105	618.0	112	262.1	101	312.8	106	45.3	91.5	50.1	97.8	95.4±4.1	94.7
F	0	370.4[a]	---	448.2	---	255.6[a]	---	310.4[a]	---	26.6[b]	---	23.7[b]	---	50.3[b]±1.6[g]	---
	25	390.6	105[c]	527.8	118[c]	273.0	107[c]	339.2	109[c]	29.0	109[c]	25.2	107[c]	54.2±2.7	108.0[c]
	50	382.0	103	504.4	112	252.9	99	283.7	91.3	23.7	89	19.8	83.8	43.5±1.8	86.6
	100	394.4	106	490.2	109	241.9	94.6	300.1	96.7	28.0	105	22.0	93.2	50.0±2.9	99.6
	210	400.4	108	483.4	108	246.8	96.6	288.8	93	30.0	113	22.0	93.2	52.0±3.2	104.0
	420	417.8	113	498.0	111	265.0	104	294.0	94.7	22.9	86.1	23.3	98.7	46.2±1.2	92.0

[a] Mean of the data from three cages is presented.

[b] Mean of the data for six animals is presented.

[c] Percent of control.

[d] Weight gain for the first week on dose.

[e] Weight gain for second week on dose.

[f] Weight gain for the entire two week dosing period.

[g] Standard error of the mean.

Week on dose	P value
1	.6412
2	.0001[a]
3	.0020[a]
4	.0529
5	.0847
6	.0001[a]
7	.0138[a]
8	.0129[a]
9	.0048[a]
10	.0314[a]
11	.0020[a]
12	.3118
13	.0376[a]

[a] Significant ($p < .05$) difference.

There is a significant dose effect for weeks 2,3,6,7,8,9,10,11, and 13. During each of these weeks the 105 mg/kg/day dose level animals consumed significantly more food than the controls. At weeks 6, 10, and 11, the 210 mg/kg/day dose level animals also consumed more food than the controls. The 420 mg/kg/day dose level animals consumed more food than the controls in weeks 6 and 11. A separate single-sample repeated-measurements analysis was run for each dose level to determine if any difference in weekly means existed. The results were:

Dose (mg/kg/day)	F[a]	DF	P value
0	854.95	12, 3	.0001[b]
105	32.16	12, 3	.0078[b]
210	8.46	12, 3	.0524
420	21.06	12, 3	.0144[b]

[a] Approximate F.
[b] Significant ($p < .05$) difference.

Table 8. Body weight data for males surviving to the 90-day sacrifice.

Week on dose	Male values (gm) at: 0 mg/kg/day	105 mg/kg/day	210 mg/kg/day	420 mg/kg/day
Start of dosing	141.0 ± 20.4[a](27)[b]	137.0 ± 19.5(30)	142.5 ± 20.4(28)	141.7 ± 25.3(28)
1	179.0 ± 22.7(27)	177.7 ± 20.9(30)	184.6 ± 20.8(28)	181.9 ± 29.5(28)
2	223.0 ± 23.1(27)	220.8 ± 21.7(30)	230.0 ± 22.0(28)	226.4 ± 31.2(28)
3	263.6 ± 23.6(27)	264.6 ± 25.2(30)	275.6 ± 25.1(28)	268.8 ± 34.1(28)
4	297.2 ± 22.6(27)	301.2 ± 21.6(30)	313.2 ± 28.0(28)	303.6 ± 35.5(28)
5	327.6 ± 23.4(27)	340.5 ± 21.0(30)	350.6 ± 31.3(28)	334.9 ± 37.4(28)
6	353.4 ± 24.6(27)	369.8 ± 23.0(30)	381.3 ± 32.9(28)	362.5 ± 39.6(28)
7	371.3 ± 27.3(27)	393.3 ± 24.7(30)	405.7 ± 33.9(28)	386.1 ± 40.8(28)
8	394.8 ± 29.4(27)	411.0 ± 25.7(30)	424.6 ± 36.4(28)	405.2 ± 42.5(28)
9	411.2 ± 32.5(27)	427.1 ± 27.3(30)	442.6 ± 40.3(28)	418.8 ± 46.0(28)
10	426.4 ± 34.8(27)	438.7 ± 28.2(30)	458.4 ± 45.8(28)	434.2 ± 48.6(28)
11	440.8 ± 36.5(27)	455.1 ± 30.3(30)	473.5 ± 48.8(28)	451.3 ± 50.1(28)
12	452.8 ± 37.6(27)	461.3 ± 29.8(30)	480.7 ± 51.5(28)	464.6 ± 51.4(28)
13[c]	464.6 ± 40.6(27)	471.8 ± 34.0(30)	492.3 ± 52.6(28)	474.9 ± 52.9(28)

[a] Mean ± SD.

[b] Number of animals.

[c] Week 13 values are the sum of weights collected on dose days 89, 90, and 91 when the animals were removed for terminal sacrifice.

Table 9. Body weight data for females surviving to the 90-day sacrifice.

Week on dose	Female values (gm) at: 0 mg/kg/day	105 mg/kg/day	210 mg/kg/day	420 mg/kg/day
Start of dosing	134.0 ± 16.9[a](30)[b]	131.6 ± 18.8(29)	134.0 ± 17.7(30)	129.9 ± 18.1(30)
1	161.1 ± 16.9(30)	158.3 ± 17.6(29)	158.9 ± 15.2(30)	155.3 ± 20.3(30)
2	182.6 ± 16.5(30)	182.2 ± 17.3(29)	180.6 ± 13.0(30)	178.1 ± 15.4(30)
3	202.1 ± 17.5(30)	201.5 ± 19.6(29)	200.9 ± 14.7(30)	198.3 ± 18.0(30)
4	219.1 ± 19.7(30)	219.1 ± 19.4(29)	217.5 ± 14.7(30)	216.0 ± 17.1(30)
5	230.0 ± 20.4(30)	231.8 ± 19.4(29)	230.1 ± 15.5(30)	228.7 ± 18.4(30)
6	240.5 ± 21.0(30)	243.8 ± 22.4(29)	241.4 ± 16.5(30)	239.1 ± 19.3(30)
7	248.1 ± 22.8(30)	251.2 ± 22.1(29)	249.2 ± 17.8(30)	246.5 ± 17.8(30)
8	254.8 ± 22.9(30)	256.7 ± 22.9(29)	254.1 ± 18.2(30)	253.3 ± 19.2(30)
9	259.9 ± 25.0(30)	261.6 ± 24.0(29)	260.0 ± 17.7(30)	259.9 ± 19.7(30)
10	266.6 ± 27.7(30)	266.9 ± 24.2(29)	265.2 ± 18.3(30)	264.6 ± 21.8(30)
11	271.0 ± 27.6(30)	270.3 ± 25.4(29)	269.6 ± 20.1(30)	269.8 ± 24.1(30)
12	273.5 ± 28.8(30)	272.0 ± 26.3(29)	272.4 ± 21.6(30)	273.9 ± 22.8(30)
13[c]	280.4 ± 28.1(30)	276.1 ± 26.3(29)	277.2 ± 20.7(30)	281.0 ± 24.5(30)

[a] Mean ± SD.

[b] Number of animals.

[c] Week 13 values are the sum of weights collected on dose days 89, 90, and 91 when the animals were removed for terminal sacrifice.

Table 10. Food consumption data for males surviving to the 90-day sacrifice.

Week on dose	Male values (g/rat/day) at: 0 mg/kg/day	105 mg/kg/day	210 mg/kg/day	420 mg/kg/day
1	18.9 ± 1.6[a](27)[b]	19.9 ± 1.0(30)	20.0 ± 2.3(28)	18.3 ± 2.3(30)
2	20.0 ± 2.3(27)	19.6 ± 0.9(30)	20.8 ± 3.6(28)	18.9 ± 2.7(29)
3	21.1 ± 3.1(27)	21.8 ± 2.4(30)	23.5 ± 6.0(28)	20.7 ± 3.3(28)
4	20.5 ± 2.0(27)	22.4 ± 2.7(30)	22.9 ± 5.6(28)	21.5 ± 2.2(28)
5	21.2 ± 3.6(27)	23.5 ± 1.7(30)	22.8 ± 3.8(28)	20.5 ± 2.1(28)
6	20.7 ± 2.7(27)	23.3 ± 2.0(30)	23.2 ± 4.1(28)	21.3 ± 2.5(28)
7	20.2 ± 2.5(27)	23.3 ± 3.4(30)	23.4 ± 4.4(28)	21.2 ± 3.3(28)
8	20.2 ± 3.8(27)	22.5 ± 2.3(30)	22.7 ± 3.5(28)	22.9 ± 2.8(28)
9	20.1 ± 3.3(27)	23.1 ± 1.9(30)	22.2 ± 4.3(28)	20.4 ± 2.9(28)
10	18.4 ± 2.7(26)[c]	22.0 ± 2.5(30)	21.7 ± 4.2(28)	19.2 ± 3.3(28)
11	20.8 ± 2.9(26)	22.5 ± 2.4(30)	21.9 ± 4.1(28)	21.1 ± 2.5(28)
12	17.1 ± 1.5(26)	21.0 ± 2.7(30)	19.1 ± 2.5(28)	19.7 ± 2.0(28)
13	17.0 ± 3.2(26)	22.5 ± 3.1(30)	19.4 ± 2.6(28)	19.2 ± 2.8(28)

[a] Mean ± SD.

[b] Number of rats.

[c] There are 26 animals used for the average instead of the 28 animals actually sacrificed because 1 cage of 2 control males (cage 14) was excluded because 1 animal in cage 14 had a bite defect.

Table 11. Food consumption data for females surviving to the 90-day sacrifice.

Week on dose	Female values (g/rat/day) at: 0 mg/kg/day	105 mg/kg/day	210 mg/kg/day	420 mg/kg/day
1	17.4 ± 1.4[a](30)[b]	18.0 ± 1.3(30)	17.4 ± 1.4(30)	17.4 ± 1.5(30)
2	15.2 ± 1.5(30)	18.4 ± 1.4(30)	16.0 ± 1.0(30)	16.3 ± 1.0(30)
3	16.9 ± 2.6(30)	20.3 ± 2.0(30)	18.9 ± 2.7(30)	18.3 ± 2.6(30)
4	18.1 ± 2.5(30)	20.6 ± 2.6(30)	18.7 ± 2.2(30)	19.3 ± 2.4(30)
5	17.6 ± 2.9(30)	19.7 ± 2.1(30)	19.0 ± 2.2(30)	19.5 ± 2.1(30)
6	17.2 ± 2.4(30)	22.3 ± 3.4(30)	20.2 ± 3.2(30)	20.2 ± 2.7(30)
7	18.2 ± 2.7(30)	21.9 ± 2.4(29)	20.0 ± 2.9(30)	19.4 ± 2.6(30)
8	16.7 ± 1.7(30)	21.1 ± 2.3(29)	19.2 ± 3.7(30)	18.5 ± 2.9(30)
9	17.8 ± 2.1(30)	22.3 ± 3.7(29)	19.4 ± 3.8(30)	19.2 ± 2.7(30)
10	17.3 ± 2.7(30)	20.0 ± 2.1(29)	19.9 ± 2.7(30)	19.1 ± 3.0(30)
11	17.0 ± 2.6(30)	20.6 ± 3.2(29)	19.7 ± 2.2(30)	19.3 ± 2.2(30)
12	16.2 ± 3.6(30)	18.0 ± 1.7(29)	17.5 ± 2.5(30)	17.6 ± 3.3(30)
13	17.4 ± 3.4(30)	20.7 ± 2.2(29)	19.8 ± 3.3(30)	19.1 ± 4.4(30)

[a] Mean ± SD.

[b] Number of rats.

Table 12. Water consumption data for males surviving to the 90-day sacrifice.

Week on dose	Male values (g/rat/day) at: 0 mg/kg/day	105 mg/kg/day	210 mg/kg/day	420 mg/kg/day
1	42.1 ± 5.3[a](28)[b]	45.6 ± 4.0(30)	48.5 ± 5.6(28)	42.5 ± 7.7(30)
2	46.0 ± 5.3(27)	45.6 ± 4.1(30)	49.3 ± 5.4(28)	45.3 ± 7.1(29)
3	45.4 ± 5.8(27)	48.5 ± 6.5(30)	49.6 ± 5.1(28)	45.1 ± 6.0(28)
4	42.9 ± 6.4(27)	48.7 ± 10.5(30)	49.1 ± 5.3(28)	45.2 ± 6.4(28)
5	44.7 ± 7.9(27)	47.5 ± 3.8(30)	50.4 ± 5.4(28)	47.3 ± 11.8(28)
6	38.3 ± 6.5(27)	41.6 ± 4.9(30)	43.9 ± 4.9(28)	41.3 ± 5.5(28)
7	39.8 ± 5.0(27)	42.6 ± 4.1(30)	45.0 ± 6.0(28)	41.7 ± 5.2(28)
8	41.2 ± 11.5(27)	43.4 ± 4.9(30)	45.4 ± 6.7(28)	44.6 ± 5.6(28)
9	45.8 ± 10.2(27)	45.1 ± 5.0(30)	49.7 ± 5.8(28)	49.1 ± 6.6(28)
10	49.8 ± 13.0(26)[c]	50.0 ± 6.0(30)	54.4 ± 10.1(28)	52.5 ± 11.0(28)
11	43.0 ± 7.9(26)	44.1 ± 4.9(30)	48.1 ± 13.9(28)	48.0 ± 6.4(28)
12	42.9 ± 13.1(26)	44.9 ± 7.1(30)	46.3 ± 8.2(28)	45.6 ± 6.9(28)
13	39.1 ± 7.5(26)	40.2 ± 5.9(30)	43.5 ± 5.3(28)	42.2 ± 6.6(28)

[a] Mean ± SD.

[b] Number of rats.

[c] There are 26 animals used for the average instead of the 28 actually sacrificed because 1 cage of 2 control males (cage 14) was excluded since 1 animal in cage 14 had a bite defect.

Table 10. Food consumption data for males surviving to the 90-day sacrifice.

Week on dose	Male values (g/rat/day) at: 0 mg/kg/day	105 mg/kg/day	210 mg/kg/day	420 mg/kg/day
1	18.9 ± 1.6[a](27)[b]	19.9 ± 1.0(30)	20.0 ± 2.3(28)	18.3 ± 2.3(30)
2	20.0 ± 2.3(27)	19.6 ± 0.9(30)	20.8 ± 3.6(28)	18.9 ± 2.7(29)
3	21.1 ± 3.1(27)	21.8 ± 2.4(30)	23.5 ± 6.0(28)	20.7 ± 3.3(28)
4	20.5 ± 2.0(27)	22.4 ± 2.7(30)	22.9 ± 5.6(28)	21.5 ± 2.2(28)
5	21.2 ± 3.6(27)	23.5 ± 1.7(30)	22.8 ± 3.8(28)	20.5 ± 2.1(28)
6	20.7 ± 2.7(27)	23.3 ± 2.0(30)	23.2 ± 4.1(28)	21.3 ± 2.5(28)
7	20.2 ± 2.5(27)	23.3 ± 3.4(30)	23.4 ± 4.4(28)	21.2 ± 3.3(28)
8	20.2 ± 3.8(27)	22.5 ± 2.3(30)	22.7 ± 3.5(28)	22.9 ± 2.8(28)
9	20.1 ± 3.3(27)	23.1 ± 1.9(30)	22.2 ± 4.3(28)	20.4 ± 2.9(28)
10	18.4 ± 2.7(26)[c]	22.0 ± 2.5(30)	21.7 ± 4.2(28)	19.2 ± 3.3(28)
11	20.8 ± 2.9(26)	22.5 ± 2.4(30)	21.9 ± 4.1(28)	21.1 ± 2.5(28)
12	17.1 ± 1.5(26)	21.0 ± 2.7(30)	19.1 ± 2.5(28)	19.7 ± 2.0(28)
13	17.0 ± 3.2(26)	22.5 ± 3.1(30)	19.4 ± 2.6(28)	19.2 ± 2.8(28)

[a] Mean ± SD.

[b] Number of rats.

[c] There are 26 animals used for the average instead of the 28 animals actually sacrificed because 1 cage of 2 control males (cage 14) was excluded because 1 animal in cage 14 had a bite defect.

FIGURE 1. Body Weight Data.
Ordinate: Body Weight (grams).

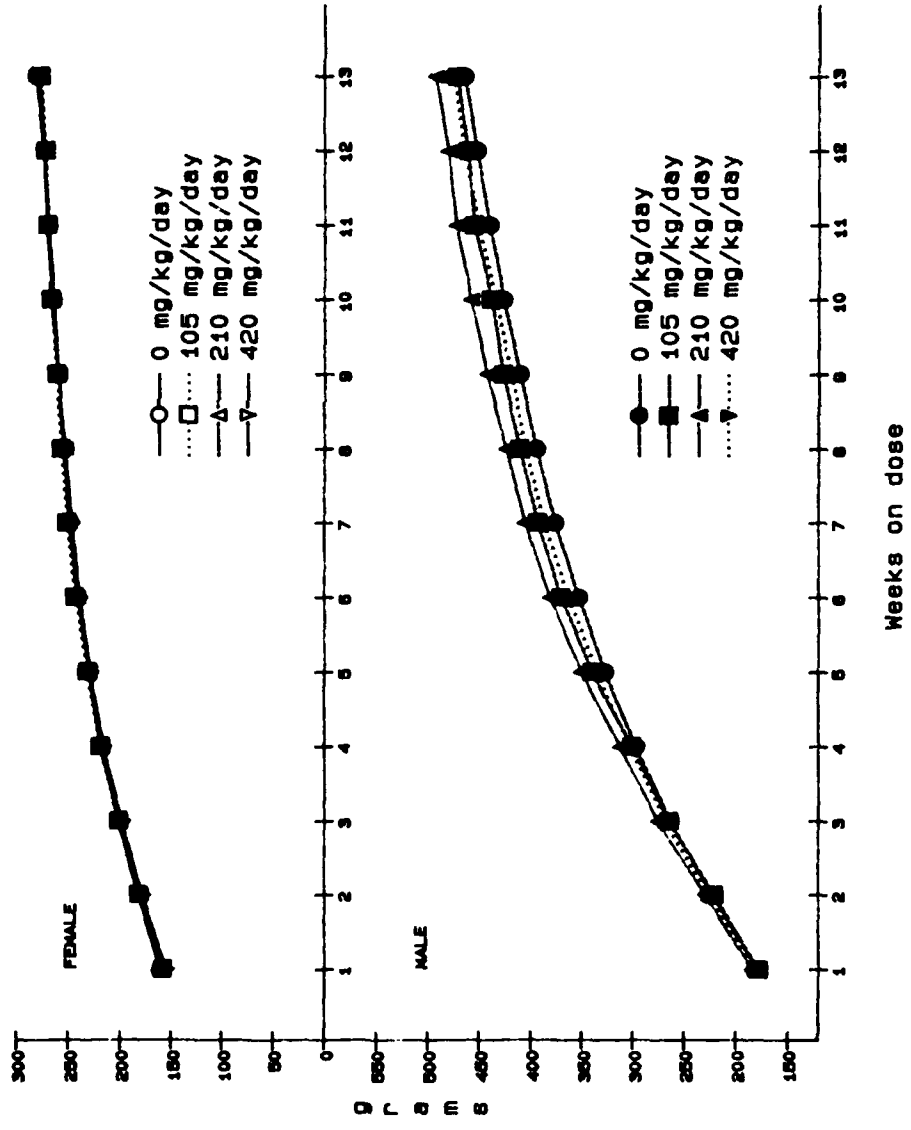


FIGURE 2. Food Consumption Data.
Ordinate: Food Consumption (g/rat/week).

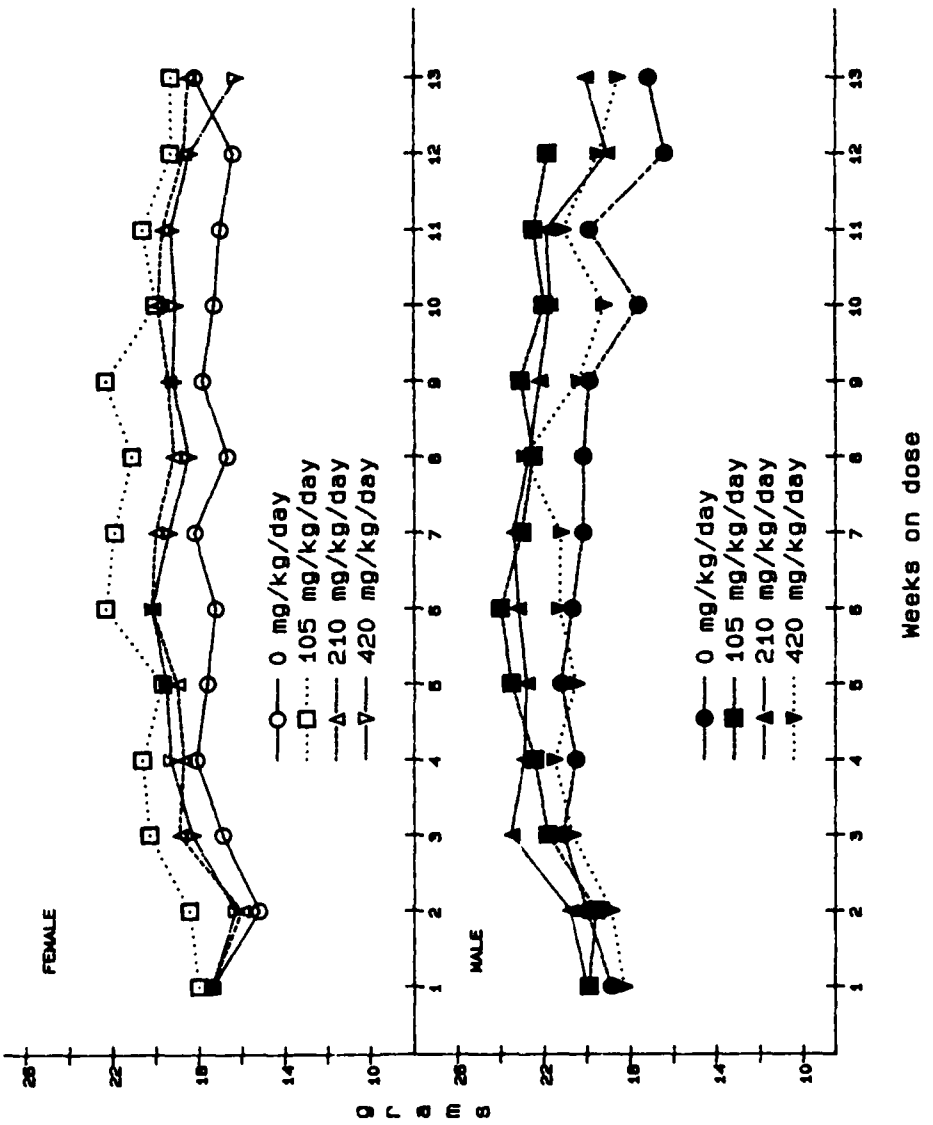
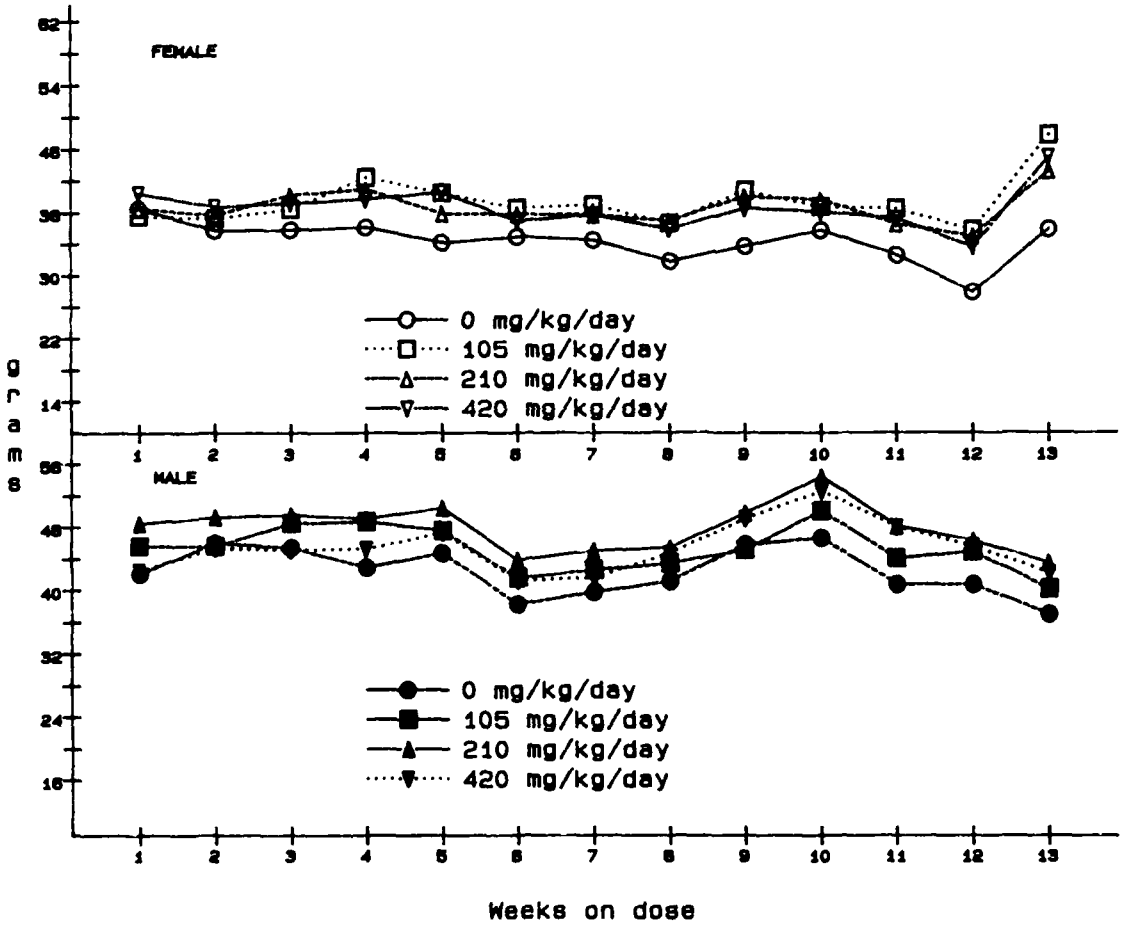


FIGURE 3. Water Consumption Data.
Ordinate: Water Consumption (g/rat/week).



The 0, 105, and 420 mg/kg/day dose levels had significant differences in their weekly means. In the statistical analyses of food consumption data in the males, the parallelism hypothesis could not be rejected. An ANOVA on food consumption indicated:

Source	DF	SS	MS[a]	F	P>F
Doses	3	.0132	.0044	1.95	.1316
Within doses	55	.1242	.0023		
Total	58	.1372			

[a] Mean Square.

There was no significant dose-related effect. The equality of the weekly means was tested and produced the following results:

Hotelling T-square	206.62
Approximate F (12,44)	206.62
Probability >F (p value)	.0001[a]

[a] Significant ($p < .05$) difference.

There was a significant difference in the weekly means. Accordingly, conclusions about food consumption in the males can only be made about a specific week.

In the statistical analyses of the water consumption data in the females, the parallelism hypothesis could not be rejected. An ANOVA on water consumption indicated:

Source	DF	SS	MS[a]	F	P>F
Doses	3	.1840	.0613	4.61	.0060[b]
Within Doses	56	.7448	.0133		
Total	59	.9288			

[a] Mean square.
[b] Significant ($p<.05$) difference.

There was a significant dose-related effect on water consumption in the females. The following Bonferroni confidence intervals compare the controls to the dose groups:

Doses being compared	Lower limit	Difference in Means	Upper limit
0 vs 105	-0.2307	-0.1268	-0.0229[a]
0 vs 210	-0.2251	-0.1212	-0.0173[a]
0 vs 420	-0.2381	-0.1342	-0.0303[a]

[a] Significant (<.05) difference.

The equality of weekly means was tested and indicated:

Hotelling T-square	2541.80
Approximate F (11, 46)	189.87
Probability >F (p value)	.0001[a]

[a] Significant (p<.05) difference).

There was a significant difference in the weekly means. Accordingly, conclusions about water consumption in the females can only be made about a specific week. In the statistical analysis of the water consumption data in the males, the parallelism hypothesis could not be rejected. An ANOVA for a dose-related effect indicated:

Source	DF	SS	Mean Square	F	P>F
Dose	3	.0178	.0059	.055	.6524
Within doses	55	.5915	.0108		
Total	58	.6093			

There was no dose-related effect. The equality of weekly means was tested and indicated:

Hotelling T-square	4172.54
Approximate F (11, 46)	278.17
Probability >F (p value)	.0001[a]

[a] Significant (p<.05) difference.

There was a significant difference in the weekly means.

2. Ophthalmologic examination. The rats' eyes were examined before the start of dosing and during week 12 on dose. No treatment-related effect was observed.

3. Organ weight. The organ weight and terminal body weight data are presented in Table 14. Six animals (Carcass identification numbers 65, 129, 131, 102, 223 and 194) were excluded from all organ weight statistical analyses because they had a lesion that would bias the results. The animals that were excluded from the statistical analyses had the following pathology:

CID[a]	Lesion	Sex	Dose level (mg/kg/day)
65	Hydronephrosis	M	210
129	Kidney malformation	M	210
131	Hydronephrosis	M	105
102	Hydronephrosis	F	420
223	Kidney mass	F	105
193	Abnormally low right kidney weight but no mention of gross pathology at necropsy.	F	105

[a] Carcass identification number.

Table 14. Organ and terminal body weight data.

Organ	Sex	Organ weight (g) and terminal body weight (g) at: 0 mg/kg/day	105 mg/kg/day	210 mg/kg/day	420 mg/kg/day	p[a]
Brain[b]	F	2.014 ± .105[c](29)[d]	2.008 ± .111(29)	1.998 ± .146(30)	1.949 ± .078(30)	0.0211[b]
Liver[b]	F	10.43 ± 1.09(29)	9.544 ± 1.051(29)	10.06 ± 1.174(30)	11.39 ± 1.416(30)	0.0003[b]
Right Kidney	F	1.046 ± .121(20)	1.089 ± .127(27)	1.103 ± .106(30)	1.069 ± .108(29)	0.5866
Left Kidney	F	1.045 ± .104(29)	1.086 ± .133(28)	1.079 ± .111(30)	1.061 ± .114(29)	0.8450
Both Kidneys	F	2.089 ± .208(29)	2.178 ± .269(27)	2.158 ± .223(30)	2.121 ± .227(29)	0.8450
Thymus	F	0.311 ± .081(29)	0.359 ± .099(29)	0.323 ± .088(30)	0.325 ± .082(30)	0.9902
Spleen[b]	F	0.579 ± .076(29)	0.647 ± .097(29)	0.586 ± .088(30)	0.558 ± .081(30)	0.0470[b]
Terminal body weight	F	235.5 ± 36.6(29)	221.9 ± 35.6(29)	225.2 ± 31.6(30)	224.4 ± 33.4(30)	
Liver	M	14.75 ± 2.641[c](28)[d]	15.54 ± 1.405(30)	16.26 ± 2.182(28)	15.79 ± 1.890(28)	0.727
Right Kidney[b]	M	1.575 ± .207(28)	1.757 ± .144(30)	1.809 ± .202(25)	1.736 ± .239(28)	0.0200[b]
Left Kidney[b]	M	1.545 ± .175(28)	1.745 ± .159(30)	1.781 ± .193(25)	1.696 ± .197(28)	0.0290[b]
Both Kidneys[b]	M	3.09 ± .351(28)	3.491 ± .319(30)	3.563 ± .385(25)	3.392 ± .394(28)	0.0290[b]
Thymus[b]	M	0.376 ± .102(28)	0.378 ± .089(30)	0.391 ± .095(28)	0.437 ± .165(28)	0.0289[b]
Spleen	M	0.682 ± .123(28)	0.802 ± .103(30)	0.794 ± .095(28)	0.698 ± .095(28)	0.6578
Brain	M	2.14 ± .166(28)	2.217 ± .127(30)	2.197 ± .114(28)	2.102 ± .137(28)	0.1131
Terminal body weight	M	385.7 ± 56.8(28)	393.4 ± 45.0(30)	415.9 ± 58.9(28)	395.6 ± 59.8(28)	

[a] P value for linear regression analysis.

[b] Significant (p.<05) difference.

[c] Mean ± SD.

[d] Number of observations.

As shown in Table 14, linear regression statistical analyses revealed a significant ($p<.05$) treatment-related effect upon the female liver, spleen and brain and upon the male kidneys and thymus. The changes in the male spleen, brain of both sexes and male thymus weights were not considered biologically significant because of the small magnitude of the changes and because only the female liver to terminal body weight ratio (see Table 15) showed a significant dose-related trend.

4. Clinical Chemistry and Hematology: The clinical chemistry (serum enzyme activities and lactate dehydrogenase isoenzymes) and the hematology (complete blood count and leukocyte differential) data are presented in Tables 16 through 23. Linear regression analyses by days on dose are presented in Tables 24 through 27. In Table 28 a summary linear regression analysis of data pooled across dates is presented. Significance is defined as $p<0.05$.

Statistical analysis (Tables 24 and 28) of the serum enzyme activities (Tables 16 and 17) identified a significant treatment-related trend in amylase activity in females. A statistically significant treatment-related trend in the pooled data for sorbitol dehydrogenase in females was observed (Table 28). Linear regression analysis of this parameter, however, across dates is not significant (Table 24). Data on total serum lactate dehydrogenase activity and electrophoretically separated isoenzymes is presented in Table 22. This enzyme is particularly subject to preanalytical sources of variation (e.g. animal stress or trace hemolysis) and results are often ambiguous. Linear regression analysis on pooled data (Table 28) is not significant. Across dates, however, linear regression analysis (Table 27) indicates several significant treatment-related trends: (1) LDH-1 at 30 days in females and at 60 days in males, (2) LDH-3 at 60 days in males, and (3) LDH-5 at 60 days in females and at 90 days in males. Only in LDH-5 in males at 90 days is the control different from the treated groups, using the latter test. In addition, LDH-5 in males at 90 days is significant in all tests at $p<0.01$. This isoenzyme is associated with liver and skeletal muscle. A decrease might suggest muscle atrophy. Inspection of the data (Table 23) with attention to the magnitude of the standard deviations reinforces the inherent variability in this enzyme parameter and the subsequent difficulty in claiming biological significance for relatively small fluctuations in activities. No clear dose-response relationship is evident.

Linear regression analyses of the data on the complete blood counts (Tables 20, 21, 26, and 28) indicate a significant difference in the white blood cell count in females at 90 days. The effect is not seen in the linear regression analysis of the pooled data and is probably a random effect. Using linear regression, a significant effect is detected in the pooled data for reticulocytes in females which, however, is not significant in the similar analysis across dates.

In summary, no clear dose-response relationships in the clinical chemistry was observed. Some quantitative variations due to animal aging, as documented in the literature, or due to logistical considerations (i.e., removal to different quarters immediately prior to sacrifice and terminal bleeding by different personnel) which may be seen in the raw data (e.g., sorbitol dehydrogenase at 90 days) are detected by the statistical analyses. While 7 parameters for females and 2 for males show statistical significance by linear regression analysis, the changes are probably random and not due to overt

Table 15. Organ to terminal body weight ratios.

Organ	Sex	Organ to body weight ratio at: 0 mg/kg/day	105 mg/kg/day	210 mg/kg/day	420 mg/kg/day	P[a]
Liver[b]	F	0.045 ± .006[c](29)[d]	0.044 ± .006(29)	0.045 ± .005(30)	0.052 ± .009(30)	0.0002[b]
Right kidney	F	0.0045 ± .0007(29)	0.0050 ± .0008(27)	0.0050 ± .0005(30)	0.0048 ± .0007(29)	0.2829
Left kidney	F	0.0045 ± .0007(29)	0.005 ± .0009(28)	0.0048 ± .0005(30	0.0048 ± .0008(29)	0.4622
Both Kidneys	F	0.0090 ± .0014(29)	0.010 ± .0018(27)	0.0097 ± .001(30)	0.0095 ± .0016(29)	0.4622
Thymus	F	0.0013 ± .0004(29)	0.0016 ± .0004(29)	0.0014 ± .0004(30)	0.0015 ± .0004(30)	0.7077
Spleen	F	0.0025 ± .0004(29)	0.0030 ± .0005(29)	0.0026 ± .0004(30)	0.025 ± .0005(30)	0.2362
Brain	F	0.0087 ± .0013(29)	0.093 ± .0015(29	0.0090 ± .0013(30)	0.0089 ± .0014(30)	0.8194
Liver	M	0.040 ± .014[c](28)[d]	0.0399 ± .0052(30)	0.039 ± .0044(28)	0.040 ± .0060(28)	0.7517
Right kidney	M	0.0042 ± .0013(28)	0.0045 ± .00046(30)	0.0044 ± .00042(25)	0.0044 ± .00071(28)	0.4766
Left kidney	M	0.0042 ± .0012(28)	0.0045 ± .00049(30)	0.0043 ± .00042(25)	0.0044 ± .00067(28)	0.5849
Both kidneys	M	0.0083 ± .0025(28)	0.0090 ± .00098(30)	0.0086 ± .00084(25)	0.0087 ± .0013(28)	0.5849
Thymus	M	0.0010 ± .0004(28)	0.00097 ± .00025(30)	0.00095 ± .00022(28)	0.0011 ± .00036(28)	0.2375
Spleen	M	0.0018 ± .0007(28)	0.0021 ± .00032(30)	0.0019 ± .00034(28)	0.0018 ± .0030(28)	0.2940
Brain	M	0.0057 ± .0014(28)	0.0057 ± .00066(30)	0.0054 ± .0008(28)	0.0054 ± .00086(28)	0.1775

[a] P value for linear regression analysis.

[b] Significant ($p<.05$) difference.

[c] Mean ± standard deviation.

[d] Number of observations.

Table 16. Clinical chemistry data for females.

Variable	Collection Period	Female values at: 0 mg/kg/day	105 mg/kg/day	210 mg/kg/day	420 mg/kg/day
Sorbitol dehydrogenase (Iu/L)	Before dosing	9.2 ± 2.6[a](6)[b]	6.8 ± 0.8(6)	7.1 ± 1.6(6)	8.9 ± 1.6(6)
	30 days on dose	16.7 ± 11.0(6)	7.7 ± 2.2(6)	9.2 ± 6.6(6)	9.3 ± 3.4(6)
	60 days on dose	7.0 ± 2.9(6)	6.7 ± 3.1(6)	5.8 ± 1.2(6)	5.6 ± 0.8(6)
	90 days on dose	20.6 ± 9.2(6)	18.3 ± 6.8(6)	18.6 ± 15.2(6)	12.4 ± 2.7(6)
Aspartate Aminotransferase (Iu/L)	Before dosing	72.5 ± 15.4(6)	97.5 ± 38.6(6)	113.5 ± 87.6(6)	91.2 ± 42.0(6)
	30 days on dose	64.7 ± 13.3(6)	66.3 ± 14.6(6)	61.8 ± 7.4(6)	57.2 ± 7.8(6)
	60 days on dose	62.5 ± 6.2(6)	86.7 ± 23.5(6)	72.5 ± 7.6(6)	67.0 ± 7.7(6)
	90 days on dose	63.0 ± 14.7(6)	51.8 ± 5.5(6)	62.2 ± 10.8(6)	55.2 ± 5.8(6)
Alanine aminotransferase (Iu/L)	Before dosing	50.5 ± 5.1(6)	51.2 ± 5.9(6)	50.5 ± 9.7(6)	47.7 ± 3.9(6)
	30 days on dose	39.2 ± 2.3(6)	36.7 ± 7.6(6)	41.3 ± 7.7(6)	33.8 ± 2.6(6)
	60 days on dose	47.2 ± 5.9(6)	51.0 ± 14.4(6)	46.5 ± 10.3(6)	45.2 ± 7.0(6)
	90 days on dose	34.7 ± 7.7(6)	25.8 ± 3.5(6)	32.2 ± 8.8(6)	33.5 ± 2.6(6)
Amylase (Iu/L)	Before dosing	992.7 ± 98.3(6)	950.0 ± 114.5(6)	967.0 ± 79.7(6)	918.5 ± 71.1(6)
	30 days on dose	1053.5 ± 115.7(6)	880.0 ± 81.2(6)	1096.0 ± 72.0(6)	1033.5 ± 74.6(6)
	60 days on dose	1026.5 ± 146.5(6)	960.0 ± 126.0(6)	989.0 ± 167.1(6)	1075.0 ± 244.7(6)
	90 days on dose	777.5 ± 242.4(6)	536.5 ± 53.1(6)	727.5 ± 193.3(6)	827.5 ± 85.6(6)

[a] Mean ± SD.
[b] Number of observations.

Table 17. Clinical chemistry data for males.

Variable	Collection Period	Male values at: 0 mg/kg/day	105 mg/kg/day	210 mg/kg/day	420 mg/kg/day
Sorbitol dehydrogenase (Iu/l)	Before dosing	11.5 ± 3.6[a](6)[b]	15.4 ± 3.0(6)	15.8 ± 4.3(6)	15.7 ± 3.8(6)
	30 days on dose	5.9 ± 1.9(5)	6.8 ± 1.6(6)	8.0 ± 5.4(6)	11.1 ± 3.4(5)
	60 days on dose	9.4 ± 9.5(5)	13.0 ± 4.4(6)	7.5 ± 2.4(6)	13.1 ± 4.2(5)
	90 days on dose	24.9 ± 9.8(4)	29.9 ± 13.2(6)	29.9 ± 3.3(6)	27.1 ± 7.6(5)
Aspartate aminotransferase (Iu/L)	Before dosing	117.2 ± 45.0(6)	94.4 ± 26.1(6)	98.2 ± 13.3(6)	117.3 ± 58.0(6)
	30 days on dose	80.2 ± 13.0(5)	65.2 ± 17.8(6)	70.5 ± 5.5(6)	65.6 ± 715.1(5)
	60 days on dose	70.8 ± 18.9(5)	67.2. ± 24.5(6)	69.5 ± 9.4(6)	69.2 ± 18.0(5)
	90 days on dose	66.8 ± 7.8(4)	58.2 ± 17.1(6)	64.0 ± 5.4(6)	63.8 ± 4.6(5)
Alanine aminotransferase (Iu/L)	Before dosing	59.2 ± 16.2(6)	62.6 ± 8.3(6)	67.5 ± 6.4(6)	66.2 ± 12.3(6)
	30 days on dose	44.2 ± 6.8(5)	39.0 ± 4.7(6)	45.5 ± 3.0(6)	47.4 ± 6.5(5)
	60 days on dose	39.8 ± 15.9(5)	43.5 ± 6.6(6)	41.7 ± 8.3(6)	49.6 ± 7.5(5)
	90 days on dose	36.8 ± 4.4(4)	29.8 ± 3.4(6)	32.3 ± 4.9(6)	39.6 ± 7.1(5)
Amylase (Iu/L)	Before dosing	912.0 ± 56.5(6)	1014.6 ± 85.0(6)	1092.0 ± 102.8(6)	992.4 ± 159.5(6)
	30 days on dose	1221.6 ± 122.6(6)	1217.5 ± 97.2(6)	1482.5 ± 424.9(6)	1471.8 ± 183.6(5)
	60 days on dose	1093.8 ± 118.1(5)	1241.5 ± 152.8(6)	1342.5 ± 270.9(6)	1080.8 ± 82.7(5)
	90 days on dose	897.8 ± 80.7(4)	761.0 ± 589.0(6)	889.0 ± 97.7(6)	975.0 ± 45.5(5)

[a] Mean ± SD.
[b] Number of observations.

Table 18. Leukocyte differential data for females. WBC: White blood cells. RBC: erythrocytes.

Variable	Collection Period	Female values at: 0 mg/kg/day	105 mg/kg/day	210 mg/kg/day	420 mg/kg/day
Neutrophil (per 100 WBC)	Before dosing	9.5 ± 3.3[a](6)[b]	7.2 ± 2.6(6)	1.5 ± 4.0(6)	11.5 ± 4.6(6)
	30 days on dose	6.3 ± 6.0(6)	7.2 ± 3.4(6)	9.3 ± 3.8(6)	10.3 ± 5.8(6)
	60 days on dose	10.7 ± 5.5(6)	9.7 ± 4.0(6)	14.8 ± 2.0(6)	14.8 ± 4.3(6)
	90 days on dose	16.5 ± 7.0(6)	17.2 ± 7.1(6)	20.0 ± 7.5(6)	14.2 ± 4.1(6)
Eosinophil (per 100 WBC)	Before dosing	1.0 ± 0.9(6)	0.0 ± 0.0(6)	0.7 ± 0.8(6)	0.0 ± 0.0(6)
	30 days on dose	0.2 ± 0.4(6)	0.8 ± 1.3(6)	0.7 ± 1.2(6)	0.0 ± 0.0(6)
	60 days on dose	0.0 ± 0.0(6)	0.5 ± 0.8(6)	0.2 ± 0.4(6)	0.0 ± 0.0(6)
	90 days on dose	0.3 ± 0.8(6)	1.3 ± 1.2(6)	0.8 ± 1.0(6)	0.5 ± 0.8(6)
Lymphocyte (per 100 WBC)	Before dosing	85.8 ± 3.6(6)	89.8 ± 3.2(6)	84.2 ± 5.0(6)	84.5 ± 3.2(6)
	30 days on dose	91.7 ± 6.8(6)	91.2 ± 4.8(6)	88.0 ± 4.8(6)	88.3 ± 6.0(6)
	60 days on dose	86.3 ± 5.1(6)	87.5 ± 3.9(6)	81.8 ± 2.4(6)	82.5 ± 5.0(6)
	90 days on dose	79.8 ± 4.9(6)	78.3 ± 7.3(6)	77.5 ± 7.9(6)	83.0 ± 3.9(6)
Monocyte (per 100 WBC)	Before dosing	3.7 ± 2.0(6)	3.0 ± 1.8(6)	3.7 ± 1.2(6)	3.7 ± 1.6(6)
	30 days on dose	1.8 ± 2.2(6)	0.8 ± 1.3(6)	2.0 ± 1.4(6)	1.3 ± 1.4(6)
	60 days on dose	3.0 ± 2.0(6)	2.3 ± 2.1(6)	3.2 ± 1.6(6)	2.7 ± 1.9(6)
	90 days on dose	3.3 ± 2.0(6)	3.2 ± 1.2(6)	1.7 ± 1.9(6)	2.3 ± 1.4(6)
Reticulocyte (per 1000 RBC)	Before dosing	5.3 ± 0.7(6)	6.2 ± 0.8(6)	5.5 ± 0.5(6)	5.2 ± 1.4(6)
	30 days on dose	1.5 ± 0.6(6)	1.5 ± 0.5(6)	1.6 ± 0.4(6)	1.9 ± 0.4(6)
	60 days on dose	1.4 ± 0.5(6)	1.7 ± 0.5(6)	1.5 ± 0.5(6)	1.9 ± 0.6(6)
	90 days on dose	1.3 ± 0.5(6)	1.0 ± 0.3(6)	1.1 ± 0.3(6)	1.2 ± 0.4(6)

[a] Mean ± SD.
[b] Number of observations.

Table 19. Leukocyte differential data for males. WBC: White blood cells. RBC: erythrocytes.

Variable	Collection Period	Male values at: 0 mg/kg/day	105 mg/kg/day	210 mg/kg/day	420 mg/kg/day
Neutrophil (per 100 WBC)	Before dosing	13.7 ± 3.7[a](6)[b]	15.3 ± 4.1(6)	15.2 ± 4.0(6)	15.3 ± 2.4(6)
	30 days on dose	9.2 ± 3.7(5)	5.5 ± 2.2(6)	7.8 ± 3.1(6)	14.0 ± 4.5(5)
	60 days on dose	13.4 ± 9.8(5)	10.0 ± 4.3(6)	11.3 ± 3.4(6)	19.8 ± 6.1(5)
	90 days on dose	18.7 ± 7.5(3)	10.0 ± 3.7(6)	15.5 ± 4.9(6)	15.0 ± 4.0(5)
Eosinophil (per 100 WBC)	Before dosing	0.3 ± 0.8(6)	1.2 ± 1.0(6)	1.2 ± 1.3(6)	1.2 ± 1.6(6)
	30 days on dose	0.0 ± 0.0(5)	0.5 ± 1.2(6)	0.3 ± 0.8(6)	0.0 ± 0.0(5)
	60 days on dose	0.0 ± 0.0(5)	0.2 ± 0.4(6)	0.8 ± 1.0 (6)	0.0 ± 0.0(5)
	90 days on dose	0.0 ± 0.0(3)	0.5 ± 0.8(6)	0.7 ± 0.8(6)	0.0 ± 0.0(5)
Lymphocyte (per 100 WBC	Before dosing	83.8 ± 4.2(6)	80.7 ± 3.8(6)	81.3 ± 5.0(6)	79.8 ± 3.4(6)
	30 days on dose	88.8 ± 4.1(5)	92.8 ± 3.2(6)	90.5 ± 3.6(6)	83.8 ± 4.6(5)
	60 days on dose	82.0 ± 12.2(5)	85.7 ± 5.3(6)	84.0 ± 4.8(6)	75.0 ± 5.1(5)
	90 days on dose	77.3 ± 3.3(6)	87.3 ± 3.3(6)	79.7 ± 6.2(6)	81.8 ± 4.3(5)
Monocyte (per 100 WBC)	Before dosing	2.2 ± 0.8(6)	2.8 ± 1.5(6)	2.3 ± 2.1(6)	3.7 ± 1.9(6)
	30 days on dose	2.0 ± 1.2(5)	1.2 ± 1.5(6)	1.3 ± 1.6(6)	2.2 ± 1.5(5)
	60 days on dose	4.4 ± 2.7(5)	4.2 ± 2.3(6)	3.8 ± 2.6(6)	5.2 ± 1.3(5)
	90 days on dose	4.0 ± 1.0(3)	2.2 ± 2.2(6)	4.2 ± 2.3(6)	3.2 ± 1.9(5)
Reticulocyte (per 1000 RBC)	Before dosing	7.4 ± 1.9(6)	8.1 ± 1.7(6)	7.7 ± 2.6(6)	5.9 ± 1.2(6)
	30 days on dose	2.1 ± 0.7(5)	1.6 ± 0.4(6)	2.1 ± 0.5(6)	1.7 ± 0.5(5)
	60 days on dose	1.9 ± 0.4(5)	1.6 ± 0.6(6)	1.3 ± 0.5(6)	1.5 ± 0.4(5)
	90 days on dose	1.3 ± 0.3(3)	1.4 ± 0.4(6)	1.5 ± 0.6(6)	1.0 ± 0.5(5)

[a] Mean ± SD.
[b] Number of observations.

Table 20. Coulter counter hematology data for females. MCH: Mean corpuscular hemoglobin. MCHC: Mean corpuscular hemoglobin content.

Variable	Collection Period	Female values at: 0 mg/kg/day	105 mg/kg/day	210 mg/kg/day	420 mg/kg/day
White Blood Cell ($10^3/mm^3$)	Before dosing	12.5 ± 1.5(6)[a,b]	11.4 ± 5.3(6)	13.4 ± 3.1(6)	11.5 ± 1.7(6)
	30 days on dose	10.0 ± 5.4(6)	11.4 ± 4.4(6)	11.4 ± 3.1(6)	12.6 ± 3.3(6)
	60 days on dose	10.8 ± 3.5(6)	10.4 ± 3.8(6)	10.4 ± 2.4(6)	10.9 ± 3.6(6)
	90 days on dose	3.4 ± 2.6(6)	7.6 ± 2.9(6)	4.2 ± 2.4(6)	9.8 ± 3.0(6)
Erythrocyte ($10^6/mm^3$)	Before dosing	5.9 ± 0.4(6)	4.9 ± 1.4(6)	5.7 ± 0.4(6)	5.8 ± 0.2(6)
	30 days on dose	6.0 ± 1.2(6)	6.3 ± 0.8(6)	6.4 ± 1.0(6)	7.0 ± 0.2(6)
	60 days on dose	6.7 ± 0.2(6)	6.3 ± 0.8(6)	6.6 ± 0.4(6)	6.7 ± 0.3(6)
	90 days on dose	5.3 ± 1.0(6)	6.5 ± 0.6(6)	5.6 ± 0.8(6)	6.9 ± 0.6(6)
Hemoglobin (g/dL)	Before dosing	14.0 ± 0.8(6)	11.8 ± 3.0(6)	13.8 ± 0.8(6)	14.1 ± 0.6(6)
	30 days on dose	13.1 ± 2.6(6)	14.5 ± 1.6(6)	14.3 ± 2.0(6)	15.6 ± 0.4(6)
	60 days on dose	14.8 ± 0.4(6)	15.3 ± 0.9(6)	14.6 ± 0.8(6)	15.4 ± 0.8(6)
	90 days on dose	11.6 ± 2.0(6)	15.4 ± 1.2(6)	12.6 ± 2.0(6)	15.5 ± 1.1(6)
Hematocrit (%)	Before dosing	40.2 ± 2.1(6)	33.7 ± 9.6(6)	39.8 ± 2.4(6)	41.0 ± 1.8(6)
	30 days on dose	38.1 ± 7.7(6)	41.6 ± 4.8(6)	41.4 ± 5.9(6)	47.0 ± 1.6(6)
	60 days on dose	40.6 ± 1.0(6)	40.0 ± 3.9(6)	41.8 ± 2.2(6)	42.1 ± 2.7(6)
	90 days on dose	32.4 ± 5.6(6)	40.8 ± 2.8(6)	35.0 ± 5.6(6)	42.6 ± 2.7(6)
Mean corpuscular volume (um^3)	Before dosing	68.2 ± 0.8(6)	67.8 ± 2.1(6)	68.7 ± 1.4(6)	69.5 ± 2.3(6)
	30 days on dose	63.3 ± 1.2(6)	66.0 ± 1.9(6)	65.2 ± 1.5(6)	67.3 ± 2.2(6)
	60 days on dose	61.0 ± 1.7(6)	64.0 ± 2.2(6)	63.3 ± 1.4(6)	63.2 ± 1.2(6)
	90 days on dose	61.3 ± 1.6(6)	63.3 ± 2.7(6)	62.7 ± 2.2(6)	62.7 ± 1.9(6)
MCH (pg/cell)	Before dosing	23.1 ± 0.4(6)	23.6 ± 1.2(6)	23.3 ± 0.5(6)	23.3 ± 0.8(6)
	30 days on dose	21.3 ± 0.7(6)	22.4 ± 0.9(6)	21.8 ± 0.5(6)	21.8 ± 0.6(6)
	60 days on dose	22.2 ± 0.7(6)	24.5 ± 2.0(6)	22.0 ± 0.6(6)	23.1 ± 0.9(6)
	90 days on dose	22.1 ± 1.0(6)	23.7 ± 0.8(6)	22.6 ± 0.5(6)	22.6 ± 0.4(6)
MCHC (g/dL)	Before dosing	33.0 ± 0.4(6)	33.7 ± 1.4(6)	32.9 ± 0.3(6)	32.6 ± 0.8(6)
	30 days on dose	33.0 ± 0.8(6)	33.4 ± 0.7(6)	33.0 ± 0.4(6)	31.7 ± 1.0(6)
	60 days on dose	36.3 ± 1.3(6)	38.2 ± 2.1(6)	34.7 ± 0.2(6)	36.5 ± 1.2(6)
	90 days on dose	35.8 ± 1.3(6)	37.4 ± 0.7(6)	35.9 ± 1.0(6)	36.1 ± 0.9(6)

[a] Mean ± SD.
[b] Number of observations.

Table 21. Coulter counter hematology data for males. MCH: Mean corpuscular hemoglobin. MCHC: Mean corpuscular hemoglobin content.

Variable	Collection Period	Male values at: 0 mg/kg/day	105 mg/kg/day	210 mg/kg/day	420 mg/kg/day
White blood cells ($10^3/mm^3$)	Before dosing	9.0 ± 2.6[a](6)[b]	9.8 ± 2.1(6)	12.0 ± 2.1(5)	10.2 ± 2.9(6)
	30 days on dose	10.5 ± 1.9(5)	11.5 ± 0.7(6)	10.2 ± 3.0(6)	14.1 ± 2.0(5)
	60 days on dose	12.1 ± 5.7(5)	11.5 ± 4.4(6)	13.5 ± 2.7(6)	13.3 ± 4.0(5)
	90 days on dose	6.8 ± 3.5(4)	6.4 ± 3.2(6)	8.7 ± 4.0(6)	5.9 ± 3.1(5)
Erythrocyte ($10^6/mm^3$)	Before dosing	5.4 ± 0.1(6)	5.4 ± 0.6(6)	5.4 ± 0.1(5)	5.5 ± 0.4(6)
	30 days on dose	6.5 ± 0.4(5)	6.5 ± 0.7(6)	6.7 ± 0.5(6)	6.7 ± 0.3(5)
	60 days on dose	7.1 ± 1.8(5)	7.5 ± 0.8(6)	7.6 ± 0.3(6)	6.9 ± 0.6(5)
	90 days on dose	6.4 ± 1.3(4)	7.3 ± 0.7(6)	7.6 ± 0.5(6)	6.8 ± 0.6(5)
Hemoglobin (g/dL)	Before dosing	13.2 ± 0.5(6)	13.0 ± 1.3(6)	13.3 ± 0.5(5)	13.2 ± 1.2(6)
	30 days on dose	15.1 ± 3.3(5)	15.1 ± 1.5(6)	15.7 ± 1.5(6)	15.4 ± 0.6(5)
	60 days on dose	14.7 ± 3.3(5)	16.1 ± 1.6(6)	16.4 ± 0.4(6)	15.0 ± 1.4(5)
	90 days on dose	13.6 ± 2.6(4)	15.4 ± 1.4(6)	15.8 ± 1.2(6)	14.8 ± 1.1(5)
Hematocrit (%)	Before dosing	38.4 ± 1.4(6)	38.2 ± 4.0(6)	38.4 ± 1.7(5)	39.2 ± 3.7(6)
	30 days on dose	41.9 ± 2.3(5)	42.2 ± 4.9(6)	43.5 ± 4.1(6)	43.5 ± 1.4(5)
	60 days on dose	43.3 ± 10.7(5)	45.2 ± 4.4(6)	46.1 ± 1.0(6)	42.5 ± 4.3(5)
	90 days on dose	38.1 ± 7.2(4)	42.8 ± 3.2(6)	44.5 ± 2.8(6)	41.9 ± 4.2(5)
Mean corpuscular volume (um^3)	Before dosing	70.3 ± 3.0(6)	70.7 ± 1.2(6)	70.4 ± 3.7(5)	71.2 ± 2.2(6)
	30 days on dose	64.2 ± 1.6(5)	65.2 ± 1.3(6)	65.2 ± 2.5(6)	65.2 ± 2.0(5)
	60 days on dose	62.0 ± 3.2(5)	61.0 ± 1.1(6)	61.2 ± 2.0(6)	62.0 ± 1.9(5)
	90 days on dose	59.8 ± 2.1(4)	59.2 ± 1.9(6)	59.3 ± 2.2(6)	61.8 ± 3.1(5)
MCH (pg/cell)	Before dosing	23.6 ± 0.7(6)	23.5 ± 0.4(6)	24.0 ± 1.3(5)	23.5 ± 0.9(6)
	30 days on dose	22.4 ± 0.8(5)	22.7 ± 0.5(6)	22.8 ± 0.9(6)	22.4 ± 0.6(5)
	60 days on dose	21.5 ± 0.9(5)	22.0 ± 0.6(6)	22.0 ± 0.9(6)	22.2 ± 1.0(5)
	90 days on dose	21.3 ± 0.8(4)	21.2 ± 0.5(6)	21.1 ± 0.8(6)	21.9 ± 0.9(5)
MCHC	Before dosing	32.8 ± 1.1(6)	32.4 ± 0.3(6)	33.1 ± 0.7(5)	32.2 ± 0.6(6)
	30 days on dose	34.2 ± 0.5(5)	34.1 ± 0.8(6)	34.4 ± 0.4(6)	33.8 ± 0.3(5)
	60 days on dose	36.0 ± 1.9(5)	37.4 ± 0.4(6)	37.2 ± 0.4(6)	37.0 ± 0.9(5)
	90 days on dose	35.5 ± 0.4(4)	35.7 ± 0.6(6)	35.3 ± 0.6(6)	35.4 ± 0.9(5)

[a] Mean ± SD.
[b] Number of observations.

Table 22. Lactic dehydrogenase electrophoresis data for females. LDH: lactic acid dehydrogenase.

Variable	Collection period	Female values at: 0 mg/kg/day	105 mg/kg/day	210 mg/kg/day	420 mg/kg/day
LDH-1 (Iu/L)	Before dosing	44.4 ± 30.6[a](5)[b]	45.8 ± 10.7(2)	88.4 ± 17.2(3)	46.9 ± 12.9(3)
	30 days on dose	51.4 ± 49.5(5)	79.6 ± 56.1(3)	54.2 ± 25.3(4)	39.9 ± 17.4(5)
	60 days on dose	23.1 ± 8.7(4)	47.0 ± 15.4(6)	25.4 ± 12.5(5)	36.3 ± 13.8(6)
	90 days on dose	20.0 ± 13.2(6)	6.3 ± 2.2(6)	13.0 ± 5.4(6)	21.7 ± 7.7(6)
LDH-2 (Iu/L)	Before dosing	28.7 ± 32.9(5)	22.9 ± 2.1(2)	90.8 ± 29.7(3)	36.2 ± 21.3(3)
	30 days on dose	19.2 ± 20.1(5)	51.6 ± 60.6(3)	29.6 ± 16.1(4)	16.4 ± 7.7(5)
	60 days on dose	9.0 ± 6.2(4)	26.4 ± 15.2(6)	16.8 ± 10.2(5)	22.9 ± 9.3(6)
	90 days on dose	11.8 ± 10.5(6)	1.9 ± 1.0(6)	3.2 ± 2.4(6)	10.6 ± 7.1(6)
LDH-3 (Iu/L)	Before dosing	26.7 ± 33.1(5)	23.5 ± 1.2(2)	100.5 ± 41.6(3)	31.5 ± 24.4(3)
	30 days on dose	16.3 ± 17.8(5)	47.3 ± 54.8(3)	31.7 ± 20.6(4)	17.3 ± 7.6(5)
	60 days on dose	18.1 ± 8.5(4)	30.4 ± 14.5(6)	39.5 ± 15.6(5)	26.4 ± 9.1(6)
	90 days on dose	13.8 ± 12.1(6)	5.2 ± 3.4(6)	4.9 ± 4.0(6)	11.5 ± 5.6(6)
LDH-4 (Iu/L)	Before dosing	37.3 ± 48.6(5)	32.8 ± 7.3(2)	133.6 ± 50.5(3)	39.1 ± 37.4(3)
	30 days on dose	24.0 ± 27.2(5)	111.8 ± 150.4(3)	55.9 ± 59.1(4)	16.7 ± 10.7(5)
	60 days on dose	30.8 ± 12.6(4)	73.4 ± 35.3(6)	77.9 ± 63.9(5)	29.1 ± 10.4(6)
	90 days on dose	22.8 ± 19.1(6)	10.2 ± 5.2(6)	19.2 ± 9.7(6)	13.6 ± 5.1(6)
LDH-5 (Iu/L)	Before dosing	127.5 ± 52.1(5)	163.6 ± 55.8(2)	206.0 ± 48.1(3)	130.9 ± 14.4(3)
	30 days on dose	270.9 ± 265.1(5)	460.0 ± 296.6(3)	327.6 ± 250.6(4)	225.0 ± 185.3(5)
	60 days on dose	454.9 ± 144.4(4)	533.6 ± 275.4(6)	550.8 ± 129.8(5)	315.8 ± 44.4(6)
	90 days on dose	158.6 ± 139.2(6)	111.0 ± 93.4(6)	209.2 ± 83.9(6)	141.2 ± 46.8(6)
LDH Total (Iu/L)	Before dosing	264.6 ± 194.2(5)	288.5 ± 77.1(5)	619.3 ± 181.7(3)	284.7 ± 104.1(3)
	30 days on dose	382.0 ± 378.9(5)	750.3 ± 586.5(5)	499.0 ± 358.1(4)	315.4 ± 226.8(5)
	60 days on dose	503.5 ± 185.4(6)	711.0 ± 294.3(6)	675.0 ± 206.2(6)	430.5 ± 64.6(6)
	90 days on dose	227.0 ± 192.4(6)	134.7 ± 101.4(6)	249.7 ± 101.0(6)	198.7 ± 61.0(6)

[a] Mean ± SD.
[b] Number of observations.

Table 23. Lactic dehydrogenase electrophoresis data for males. LDH: lactic acid dehydrogenase.

Variable	Collection period	Male values at: 0 mg/kg/day	105 mg/kg/day	210 mg/kg/day	420 mg/kg/day
LDH-1 (Iu/L)	Before dosing	15.6 ± 5.2[a](3)[b]	17.5 ± 4.8(4)	13.8 ± 5.2(5)	24.1 ± 17.4(4)
	30 days on dose	35.8 ± 14.2(5)	22.0 ± 9.7(5)	25.3 ± 10.1(6)	28.4 ± 29.0(5)
	60 days on dose	28.0 ± 11.9(5)	24.1 ± 14.2(6)	32.9 ± 11.2(6)	21.7 ± 22.8(5)
	90 days on dose	13.8 ± 4.4(4)	9.0 ± 5.7(6)	11.8 ± 6.5(6)	13.7 ± 6.3(5)
LDH-2 (Iu/L)	Before dosing	10.4 ± 1.6(3)	17.5 ± 5.1(4)	13.6 ± 5.1(5)	18.6 ± 18.8(4)
	30 days on dose	20.0 ± 10.8(5)	15.1 ± 9.4(5)	17.4 ± 7.6(6)	14.8 ± 14.8(5)
	60 days on dose	15.5 ± 8.5(5)	11.8 ± 8.8(6)	20.9 ± 9.7(6)	10.9 ± 9.8(5)
	90 days on dose	4.4 ± 1.4(4)	4.6 ± 2.9(6)	4.1 ± 3.6(6)	6.0 ± 2.3(5)
LDH-3 (Iu/L)	Before dosing	13.1 ± 3.5(3)	25.2 ± 14.1(4)	13.0 ± 7.6(5)	29.3 ± 28.9(4)
	30 days on dose	23.8 ± 11.1(5)	20.5 ± 14.9(5)	18.1 ± 8.1(6)	16.3 ± 17.4(5)
	60 days on dose	28.1 ± 11.4(5)	15.0 ± 8.7(6)	25.0 ± 11.1(6)	16.0 ± 8.8(5)
	90 days on dose	7.6 ± 2.1(4)	7.2 ± 6.2(6)	5.2 ± 3.8(6)	8.1 ± 5.2(5)
LDH-4 (Iu/L)	Before dosing	11.7 ± 6.0(3)	31.9 ± 18.0(4)	15.3 ± 8.9(5)	47.1 ± 58.3(4)
	30 days on dose	31.7 ± 11.0(5)	32.2 ± 23.5(5)	23.0 ± 9.8(6)	21.9 ± 25.0(5)
	60 days on dose	37.0 ± 13.9(5)	22.2 ± 11.2(6)	49.0 ± 13.3(6)	27.1 ± 24.1(5)
	90 days on dose	10.1 ± 3.1(4)	13.2 ± 12.6(6)	7.9 ± 5.0(6)	12.3 ± 10.1(5)
LDH-5 (Iu/L)	Before dosing	84.5 ± 49.9(3)	104.2 ± 39.1(4)	74.7 ± 25.1(5)	126.1 ± 58.5(4)
	30 days on dose	442.7 ± 333.4(5)	217.8 ± 121.5(5)	258.1 ± 132.6(6)	190.0 ± 192.9(5)
	60 days on dose	378.6 ± 185.6(5)	206.6 ± 79.3(6)	400.0 ± 211.6(6)	293.9 ± 127.0(5)
	90 days on dose	162.8 ± 126.2(4)	107.2 ± 39.5(6)	92.0 ± 36.9(6)	94.1 ± 87.1(5)
LDH total (Iu/L)	Before dosing	135.3 ± 62.6(3)	196.2 ± 77.4(4)	130.4 ± 44.5(5)	245.2 ± 177.8(4)
	30 days on dose	554.0 ± 328.3(5)	364.3 ± 206.9(6)	342.0 ± 158.0(6)	271.4 ± 278.6(5)
	60 days on dose	487.2 ± 204.0(5)	279.8 ± 114.3(6)	527.7 ± 241.2(6)	369.6 ± 180.5(5)
	90 days on dose	198.8 ± 129.2(4)	141.3 ± 56.3(6)	121.0 ± 42.6(6)	134.2 ± 109.9(5)

[a] Mean ± SD.
[b] Number of observations.

Table 24. Summary of linear regression analyses on the clinical chemistry data.

Variable	Collection Period	P value for linear regression analyses for: Value for Collection Period Minus Before-Dosing Value		Value for Collection Period Divided by Before-Dosing Value	
		F	M	F	M
Sorbitol dehydrogenase	30 days on dose	0.1179	0.8963	0.1546	0.4771
	60 days on dose	0.3574	0.2942	0.2160	0.3871
	90 days on dose	0.1388	0.9729	0.1939	0.6887
Aspartate aminotransferase	30 days on dose	0.3926	0.2250	0.1678	0.5112
	60 days on dose	0.5525	0.2019	0.5013	0.3356
	90 days on dose	0.5456	0.1037	0.3370	0.1095
Alanine amino-transferase	30 days on dose	0.7137	0.3240	0.4511	0.7086
	60 days on dose	0.9623	0.4480	0.9835	0.9443
	90 days on dose	0.3371	0.4060	0.2758	0.8491
Amylase	30 days on dose	0.0601	0.3051	0.0431[a]	0.4115
	60 days on dose	0.1819	0.6736	0.1507	0.5925
	90 days on dose	0.0549	0.4320	0.0467[a]	0.2907

[a] Significant ($p<.05$) treatment-related trend.

Table 25. Summary of linear regression analyses on the blood differential data.

Variable	Collection Period	P value for linear regression analyses for: Value for Collection Period Minus Before-Dosing Value		Value for Collection Period Divided by Before-Dosing Value	
		F	M	F	M
Neutrophil	30 days on dose	0.7501	0.1346	0.3906	0.0700
	60 days on dose	0.4832	0.0997	0.4982	0.1065
	90 days on dose	0.1699	0.8382	0.1432	0.7083
Eosinophil	30 days on dose	0.5575	0.2522	-[a]	-[a]
	60 days on dose	0.2295	0.3826	-	-
	90 days on dose	0.4223	0.3780	-	-
Lymphocyte	30 days on dose	0.7795	0.3789	0.7998	0.4455
	60 days on dose	0.4478	0.2612	0.4505	0.2641
	90 days on dose	0.1192	0.4640	0.1200	0.4645
Monocyte	30 days on dose	0.7681	0.1727	-[a]	-[a]
	60 days on dose	0.8045	0.5744	-	-
	90 days on dose	0.2931	0.2917	-	-
Reticulocyte	30 days on dose	0.1859	0.2907	0.0531	0.9940
	60 days on dose	0.1258	0.3079	0.0598	0.8183
	90 days on dose	0.6178	0.3135	0.6518	0.7325

[a] These values are blank (-) because division by zero is impossible.

Table 26. Summary of linear regression analyses on the Coulter Counter hematology data. MCH: Mean corpuscular hemoglobin. MCHC: Mean corpuscular hemoglobin content.

Variable	Collection Period	P value for linear regression analyses for: Value for Collection Period Minus Before-Dosing Value		Value for Collection Period Divided by Before-Dosing Value	
		F	M	F	M
White blood cell	30 days on dose	0.2376	0.9359	0.6313	0.5816
	60 days on dose	0.7479	0.6668	0.9483	0.3019
	90 days on dose	0.0426[a]	0.2371	0.3294	0.4472
Erythrocyte	30 days on dose	0.3239	0.9436	0.7848	0.8014
	60 days on dose	0.7603	0.4559	0.6227	0.4249
	90 days on dose	0.2357	0.8351	0.6937	0.7963
Hemoglobin	30 days on dose	0.3321	0.8852	0.6937	0.7963
	60 days on dose	0.7707	0.6534	0.6237	0.5970
	90 days on dose	0.3001	0.9217	0.6559	0.9596
Hematocrit	30 days on dose	0.2248	0.8427	0.6450	0.7586
	60 days on dose	0.7861	0.4442	0.6419	0.3855
	90 days on dose	0.3247	0.8673	0.7155	0.8650
Mean corpuscular volume	30 days on dose	0.1095	0.9907	0.0916	0.9555
	60 days on dose	0.9472	0.7784	0.9386	0.8002
	90 days on dose	0.5655	0.1955	0.6446	0.1409
MCH	30 days on dose	0.8148	0.7678	0.8052	0.7995
	60 days on dose	0.9562	0.2934	0.9493	0.2494
	90 days on dose	0.9766	0.1252	0.9702	0.0870
MCHC	30 days on dose	0.1659	0.8516	0.1524	0.8368
	60 days on dose	0.9497	0.2729	0.9972	0.2740
	90 days on dose	0.2248	0.9709	0.4973	0.9957

[a] Significant ($p<.05$) treatment-related trend.

Table 27. Summary of linear regression analyses on the lactic dehydrogenase (LDH) electrophoresis data.

Variable	Collection Period	P value for linear regression analyses for: Value for Collection Period Minus Before-Dosing Value		Value for Collection Period Divided by Before-Dosing Value	
		F	M	F	M
LDH-1	30 days on dose	0.0355[a]	0.4539	0.0573	0.3079
	60 days on dose	0.7236	0.0147[a]	0.8559	0.0664
	90 days on dose	0.7313	0.2722	0.4781	0.6557
LDH-2	30 days on dose	0.0844	0.6727	0.1346	0.1175
	60 days on dose	0.8837	0.0828	0.8935	0.1663
	90 days on dose	0.5293	0.3849	0.2931	0.7219
LDH-3	30 days on dose	0.1528	0.7241	0.1717	0.3853
	60 days on dose	0.9592	0.0273[a]	0.9402	0.0796
	90 days on dose	0.5728	0.216	0.3066	0.8238
LDH-4	30 days on dose	0.2499	0.3786	0.1466	0.6854
	60 days on dose	0.9582	0.0615	0.7280	0.1564
	90 days on dose	0.5833	0.1060	0.2684	0.2860
LDH-5	30 days on dose	0.2710	0.3544	0.1350	0.3728
	60 days on dose	0.1162	0.1295	0.0450[a]	0.1796
	90 days on dose	0.8523	0.0010[a]	0.5636	0.0090[a]

[a] Significant ($p<.05$) treatment-related trend.

Table 28. Summary of linear regression analyses on the pooled 30-, 60-, and 90-day on dose data. LDH: lactic dehydrogenase. WBC: white blood cell. RBC: erythrocyte. MCH: Mean corpuscular hemoglobin. MCHC: Mean corpuscular hemoglobin content.

Variable	P value for linear regression analyses for: Average of 30-, 60-, and 90-day Data Minus Before-Dosing Value		Average of 30-, 60-, and 90-day Data Divided by Before-Dosing Value	
	F	M	F	M
LDH-1	0.4600	0.3513	0.2689	0.6973
LDH-2	0.5388	0.4381	0.3023	0.9175
LDH-3	0.6012	0.1866	0.3940	0.5035
LDH-4	0.5942	0.1223	0.3230	0.3452
LDH-5	0.2527	0.0875	0.1782	0.0899
Neutrophil	0.7871	0.1844	0.8246[b]	0.1970[b]
Eosinophil	0.2993	0.2635	-[b]	-[b]
Lymphocyte	0.7542	0.5025	0.7554[b]	0.5292[b]
Monocyte	0.4875	0.2215	-[b]	-[b]
Reticulocyte	0.2484	0.2965	0.0340[a]	0.9379
WBC	0.1318	0.3163	0.5688	0.2058
RBC	0.4189	0.4899	0.9488	0.4282
Hemoglobin	0.4614	0.7125	0.8888	0.6460
Hematocrit	0.4204	0.5272	0.9001	0.4789
Mean corpuscular volume	0.7404	0.9757	0.6449	0.9235
MCH	0.9213	0.3074	0.9144	0.2485
MCHC	0.7796	0.3979	0.8344	0.3971
Sorbitol dehydrogenase	0.0267[a]	0.8266	0.0476[a]	0.9576
Aspartate aminotransferase	0.4773	0.1954	0.2646	0.3037
Alanine aminotransferase	0.7770	0.2317	0.8546	0.6410
Amylase	0.0481[a]	0.7630	0.0367[a]	0.9711

[a] Significant ($p<.05$) treatment-related trend.
[b] These values are blank because division by zero is impossible.

toxicity of 1,4,-dithiane. Among these parameters a change of at least 1 or 2 fold is usually considered necessary for biological significance.

5. Pathology. The computer-generated pathology summaries are stored in the NCTR Archives. The following seven animals died before the 90-day sacrifice and were excluded from all statistical analyses:

CID	Sex	Dose level (mg/kg/day)	Days on dose	Condition at arrival for necropsy
1	M	210	1	Dead
2	M	210	1	Dead
3	M	0	3	Dead
4	M	420	7	Dead
5	M	420	12	Dead
6	F	105	51	Moribund
7	M	0	62	Dead

Gavage accident was listed as the cause of death in 6 of the 7 rats. Animal number seven's cause of death was listed as "unknown." However, this animal did have lesions in its lungs. Histopathologic examination of these seven animals was limited to the nose and the only animal to have anisotropic crystals in the nasal olfactory mucosa was the male dosed with 420 mg/kg/day of 1,4-dithiane for seven days (CID Number 4.)

The microscopic lesions observed in the high dose groups (420 mg/kg/day) and in some instances, in the lower dose groups that were attributed to 1,4-dithiane involved three organs: nose, liver and kidney.

The nasal lesions, crystals and granulomatous inflammation, were present in both sexes (Table 29). The severity was the greatest in high and intermediate dose groups of both sexes. In the low dose groups at 90 days, these dose-related ($p<.00005$) lesions were present in 2/30, (6.7%) males and 24/29, (82.8%) females. The severity of the lesion, as measured by the number of crystals and the degree of inflammation, was much less in the low dose group. The lesion consisted of the presence of anisotropic crystals bilaterally distributed in the olfactory mucosa of the turbinates and nasal septum. These lesions were the severest in the posterior portion of the nasal cavity, possibly because the olfactory mucosa are the predominant covering of this area. The lesions were not observed in nasal respiratory epithelial mucosa of any animal. The crystals varied widely in size and shape. Many were extremely large while others were considerably smaller, perhaps fragments of the larger crystalline deposits. The crystals were present in the olfactory epithelium and dispersed between the glands and nerves of the lamina propria. Wherever the crystals were present in the mucosa, there was an accompanying granulomatous inflammatory reaction. This was characterized by phagacytosis of the crystals by multinucleated giant cells. The crystals were apparent when viewed with tungsten light but were very brilliant when viewed with polarized light. These crystals, in some cases, also induced focal osseous and cartilagenous inflammation and degeneration where they were closely associated with bone and cartilage. In some instances the crystals extended beyond the mucosa into the nasal cavity and in these instances there was a prominent accumulation of neutrophils around the crystals. The crystals were observed only once outside the nasal cavity and they were present in

association with a chronic inflammatory urethritis (animal 236). This animal also had chronic cystitis and chronic active inflammation in the kidneys. A

Table 29. Incidence of 1,4-dithiane-induced lesions in animals surviving to the terminal (90-day) sacrifice.

Lesion	Sex	Dose (mg/kg/day) 0	105	210	420
Nasal crystals	M	0/28[a](0)[b]	2/30(6.7)	28/28(100)	28/28(100)
	F	0/30(0)	24/29(82.8)	30/30(100)	30/30(100)
Hypertrophy of centrilobular hepatocytes	F	0/30(0)	0/29(0)	0/30(0)	26/30(86.7)
Cytoplasmic vacuolation of hepatocytes	F	0/30(0)	0/29(0)	0/30(0)	7/30(23.3)
Eosinophilic cytoplasmic renal granules	M	0/28(0)	0/30(0)	0/28(0)	26/28(92.8)

a: Number of animals with the lesion over the total number of the animals in the treatment group.
b. Incidence as a percent.

few crystals were also present in the renal pelvis of this animal, perhaps the result of reflux.

The liver lesions associated with the administration of 1,4-dithiane were limited to females and almost exclusively to those in the 420 mg/kg/day group (Table 29). The changes were minimal to mild in their severity. One of these changes consisted of hypertrophy of the centrilobular hepatocytes that caused a minimal amount of distortion of lobular architecture. This change was observed in 26 of 30 (86.7%) high dose females. The other lesion seen in the livers of 7 (23.3%) of the high dose females was cytoplasmic vacuolation of hepatocytes in the periportal region of the lobules. This change was also observed in two additional females at lower doses: one each in the intermediate and low dose groups.

The renal lesion associated with the administration of 1,4-dithiane was observed in 26 of 28 males (92.8%) in the high dose group (Table 29). This lesion was not present in the kidneys of the males in the intermediate dose group. The lesion was diagnosed as a cytoplasmic alteration which was a diffuse change throughout the renal cortex that was characterized by multiple eosinophilic granules or droplets of varying size in the cytoplasm of the convoluted tubules. The distal convoluted tubules appeared to be the most commonly involved.

In summary, four distinct 1,4-dithiane treatment-related lesions were present in three organs. In the liver, predominantly in the high dose females, there was minimal centrilobular hepatocellular hypertrophy and cytoplasmic vacuolation of the periportal hepatocytes. The reason for these minimal changes is not understood except that it is morphological evidence that this organ is responding to the toxicity of 1,4-dithiane. The kidneys of the high dose males also exhibited a 1,4-dithiane-related lesion which was

eosinophilic cytoplasmic droplets or granules. These droplets or granules were probably products of either cellular absorption or excretion. These droplets were only observed in the cortical convoluted tubules and their importance, like those of the liver lesions, is not known. The most interesting and certainly the most unusual lesion was the presence of anisotropic crystals in olfactory mucosa of the nasal cavity. This type of lesion has not been previously reported in computer-assisted literature searches. It is well known that this tissue is biologically very active and capable of metabolizing many xenobiotics. The only finding that was revealed by this 90-day study was their presence and the assumption that they caused a granulomatous inflammatory reaction. The identification of these crystals was not accomplished and it is not understood how or why they were formed in this particular tissue. The fact that 1,4-dithiane is a sublimable, volatile and a very odoriferous chemical may have great significance in the eventual understanding of this crystallization. The crystallization in the olfactory nasal mucosa is the major observation of this 90-day study and additional studies directed towards the understanding of this lesion are certainly warranted.

V. DISCUSSION

The deposition of anistropic crystals in the olfactory nasal mucosa of both sexes at all dose levels is the most significant pathologic finding in this 90-day study. Giddens (1972) reported that the olfactory mucosa of the DF mouse was most sensitive to inhaled, very water soluble SO_2. Giddens (1972) suggested that the SO_2-induced damage might be intensified due to SO_2-induced nasal exudates which could diminish nasal airway patency and increase SO_2 absorption. Buckley et al (1985) reported that dimethylamine in a twelve-month inhalation study produced pathologic changes in both the respiratory epithelium in the anterior nasal passages and in the olfactory epithelium, especially that lining the anterior dorsal meatus. Dimethylamine is highly water-soluble (55% by weight at 25°C) and, accordingly, would readily be absorbed by nasal surface secretions. The importance of water solubility for respiratory tract lesions has been discussed by Buckley et al. (1984). Morgan et al. (1986, 1986a) reported the effects of acute and chronic formaldehyde inhalation experiments in Fischer 344 rats and suggested that both regional exposure and local tissue susceptibility may account for the distribution of formaldehyde - induced tumors. In contrast to the high water solubility of SO_2, dimethylamine, and formaldehyde, 1,4-dithiane is essentially insoluble in water. This insolubility makes 1,4-dithiane unusual among nasal irritants. Also, 1,4-dithiane is unique in that it caused deposition of anisotropic crystals in only the 1,4-dithiane-treated animals. 1,4-dithiane is the only compound known to cause deposition of anisotropic crystals in the turbinates. There was a very strong odor in the animal room for 4 to 5 hours after the daily gavagings. Accordingly, it is possible that some 1,4-dithiane was inhaled. However, the anistropic crystals were located within the mucosa of only treated animals and were probably deposited by some unknown mechanism after systemic absorption of the 1,4-dithiane given by gavage. The Material Safety Data Sheet supplied by Fairfield Chemical Co. (see copy in Appendix 1) reports that vapors of 1,4-dithiane are irritating to the human nose and human upper respiratory tract. It is unknown if 1,4-dithiane causes crystal deposition in the human nose. However, it is interesting that the human nose is sensitive to 1,4-dithiane and that the rat nose exhibits crystal deposition after dosing with 1,4-dithiane.

In view of the pathologic lesions in the nose, liver, and kidney observed in the 90-day study, future work is indicated in the following areas:

1.) Determining the chemical composition of the crystals. Enzymes could be used to digest the organic mucosal material and a subsequent sucrose-gradient density procedure could separate the crystals from the organic material. Thereafter, various chemical procedures could be used to chemically characterize the crystals.

2.) Studying the time needed for deposition of the nose crystals. A male dosed with 420 mg/kg/day for seven days had the crystals but another male on the same dose for 12 days did not have the nose crystals. The time required for deposition of the nose crystals is important for a risk assessment analysis for 1,4-dithiane.

3) Studying the reversibility or persistence of the nose crystals by stopping dosing and killing at various times thereafter. The nose crystals were associated with a granulomatous inflammation that may progress to a more severe chronic rhinitis and, possibly, to carcinoma. Determining the progression or regression of the 1,4-dithiane-induced tissue damage is essential for a risk-assessment analysis for 1,4-dithiane.

4.) Studying the strain and species specificity of crystal deposition by dosing F-344 rats and B_6C_3F1 mice (the rodent strains used by NTP in its bioassays) and by dosing BALB/C mice (the mouse strain used in the 24,000 ED_{01} "megamouse" study conducted at NCTR to determine a 1% carcinogenic dose for 2-acetylaminofluorene). While unlikely, it is possible that the CD rat used in this study is unique in its response to 1,4-dithiane administration. Determining the crystal deposition, if any, in other species and strains is essential for a risk-assessment analysis of 1,4-dithiane. The rodent is an obligatory nasal breather. It may be prudent to test an oronasal species, e.g., a rabbit.

5.) Studying other compounds (e.g., 1,4-thioxane or other mustard metabolites or 1,3-dithiane) for crystal deposition. It is of scientific interest to know if close chemical congeners (e.g., 1,3-dithiane) of 1,4-dithiane would also deposit nose crystals.

6.) Determining the ultrastructure of the crystals and granules by electron microscopy.

7.) Performing a metabolism study in the CD rat. In view of the uniqueness of the nasal crystals, a determination of the metabolites of 1,4-dithiane is mandated if an understanding of the mechanism responsible for the crystal deposition is to be achieved. Knowledge of the metabolites of 1,4-dithiane and of the composition of the crystals can be tied together by a study of the pharmacokinetics of 1,4-dithiane to establish a temporal framework for these transformations.

A suggested drinking water calculation was performed (see XII. DRINKING WATER CRITERION). The suggested drinking water criterion was calculated to be 2.45 mg of 1,4-dithiane/liter of water.

VI. LOCATION FOR SPECIMENS AND RAW DATA

The location for the animal husbandry, analytical chemistry, microbiology and pathology records or specimens is given in Table 30. The computer tape containing the toxicology (weights and observations) and pathology (microscopic) data is in Building 5 Computer Center. The manual records and other records for the range-finding study are in the NCTR archives. The disk (PNCT-21) which is used for statistical analyses is also in the Building 5 Computer Center. The Final Report is located in Building 5D, Room 189 (the NCTR Quality Assurance Archives).

Table 30. Location of specimens and raw data.

Support Element	Location	
	Building	Room
Animal Husbandry	15	114a
Analytical Chemistry	13	118
(Assay records, notebooks)	51	109
Bldg. 51, Room 110 is the Chemistry Archives		
Microbiology	61	Various labs
Dose Preparation	5D	167
Pathology		
Blocks (491-1 to 491-240) and slides (491-1 to 491-240)	5B	130
Wet tissues	5B	158
Gross and microscopic forms	5B	121C
ORIMS	Stored on database (5C-112)	
Comparative Toxicology	5D	Schieferstein's office

The 1,4-dithiane and sesame oil left over at the end of the 90-day study is stored in Diet Preparation (Building 5).

VII. ACKNOWLEDGMENTS

In order to accomplish a task of the magnitude of the 14- and 90-day studies, the cooperative efforts of all the support divisions at NCTR were required. The Principal Investigator would like to take this opportunity to thank them collectively. I would like to specifically thank the following divisions and people:

Animal Husbandry for breeding, allocating, caring for, dosing, collecting the predosing and 30- and 60-day clinical chemistry samples and removing animals for necropsy (W. McCallum, P. Albright, M. Moore and the Bionetic's and government caretakers);

Analytical Chemistry for developing analytical methodology to quantitate 1,4-dithiane solutions, for recrystallizing the 1,4-dithiane, for preparing 1,4-dithiane solutions, for determining 1,4-dithiane concentrations, for help in the unsuccessful attempt to chemically characterize the nasal crystals and for GC/MS and NMR spectra (D. Nestorick, P. Freeman, D. Miller and J. Lay);

Microbiology for surveillance of the NCTR breeding colony (J. Tortorich);

Diet Preparation for supplying animal feed (R. Loe, R. Smith and the Bio Serv. employees);

Pathology Services for necropsy of the animals, block and slide preparation, pathologic evaluation of the slides, clinical chemistry, and ophthalmologic examination (O. Robinson, W. Sheldon, J. Crowell, B. Johnson, A. Warbritton, and the other members of the PAI staff, and R. Munger);

Toxicology Data Management Services for breeding information, allocation/randomization procedure, for data collection, storage and for assistance in the statistical analyses (J. Appleget, K. Carroll, C. Kolafa, and S. Cantrell);

Biometry for data analyses and interpretation of the analyses (R. Allen, S. Cantrell);

General Services Branch and PAI for preparing slides, tables and figures for the research review, SOT presentation and publication (N. Aston, R. Barringer, R. Parker and A. Warbritton);

Management at all levels for advice and support (S. Douglas, H. Wiley, D. Brand, P. Attwood, N. Littlefield, and C. Jackson);

Safety for their help in mask fitting and answering personnel questions (T. Cole and C. Partridge); and

Quality Assurance for performing GLP audits (D. Taylor, A. Swartzentruber and J. Berky).

I want to thank a former NCTR colleague (K. Morgan, now with CIIT) for reviewing slides of the turbinates of 1,4-dithiane-treated rats and for his helpful discussion of this study's results.

Finally, I want to thank the Division of Comparative Toxicology's secretary (R. York) for typing the monthly reports and the other correspondence associated with the project and the Division of Chemistry's secretary (L. Davis) and the Division of Animal Husbandry's secretary (K. Miller) for typing the Final Report.

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IX. APPENDIX I.

SOCIETY OF TOXICOLOGY

ABSTRACT FORM FOR THE 1987 ANNUAL MEETING

Any correspondence regarding your abstract *must* reference the above number.

Follow format for heading. Leave double space between heading and abstract. Text of abstract *must* be single spaced. There will be a charge of $25 for any abstract that must be retyped. Leave no margins — type *must* be within rectangle. Single space, elite type, except for double space between body of text and authors. Deadline for receipt in Executive Secretary's office is October 24, 1986. Follow instructions accompanying this form. Submit original and one copy only. Original *must* be on this form. Proof carefully.

SUBCHRONIC TOXICITY STUDY OF 1,4-DITHIANE IN THE CD RAT. G.J. Schieferstein, G. Reddy, W.G. Sheldon, and S.A. Cantrell. National Center for Toxicological Research, Jefferson, AR, US ARMY Medical Bioengineering R&D Laboratory, Fort Detrick, Frederick, MD

There is a need for toxicological information on 1,4-dithiane, an environmental contaminant found in and around locations where mustard gas (bis-[chloroethyl]sulfide) has been disposed. In a 90-day rat subchronic study, in which CD strain rats were dosed by gavage at 0, 105, 210, and 420 mg/kg/day (30 rats/sex/dose group), no overt toxicity, treatment-related mortality or treatment-related clinical chemical, hematologic, or ophthalmologic changes were found. The female livers and the male kidneys were significantly heavier ($p < 0.05$) in the treated animals. Anisotropic crystals of undetermined chemical composition were deposited in the olfactory mucosa of both sexes. These crystals were present in similar amounts in both sexes of the high and intermediate dose groups. In the low dose group, the crystals were present in greater amounts in the females. The crystals were not observed in the control animals. Other treatment-related manifestations were eosinophilic cytoplasmic granulation of the convoluted renal tubule cells in the high dose males and minimal hypertrophy of the centrilobular region of the liver in the high dose females. (Supported by Army Medical Research & Development Command Project Order 85PP5870).

1. Name and address of presenting author:

Name: George J. Schieferstein

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Address:

Jefferson, AR 72079
City State Zip Code

USA
Country

Telephone (501) 541-4365

2. Membership status: _x_ SOT member ____ Non-member
Abstracts must have an SOT member as an author or be sponsored by a member. If sponsored, the SOT member sponsor must sign below:

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____ 6. Genotox/Mutagenesis
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____ 8. Reproductive/Teratology
____ 9. Cardiovascular/Renal
____ 10. Inhalation
____ 11. Hematologic
____ 12. Dermal/Ocular
____ 13. Hepatic/GI system
Nervous system:
____ 14. Neurochemistry
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MATERIAL SAFETY DATA SHEET

Fairfield Chemical Company, Inc.
Post Office Box 20
Blythewood, SC 29016

Emergency Phone Number: (803)754-3856

SECTION I - PRODUCT IDENTIFICATION

Product Name: 1,4-Dithiane
FCC Catalog #: D-819000
(Synonyms):
Formula Weight: 120.24
Formula: $C_4H_8S_2$

Hazardous Components: 1,4-Dithiane: STENCH

SECTION II - PHYSICAL DATA

Boiling Point: Not Known
Melting Point: 110-112°
Vapor Pressure (mm): Not Known
Appearance: White crystals
SP Gravity: N/A
% Volatile, by Volume: 100
Water Solubility: Insoluble
Refractive Index (20C): N/A

SECTION III - FIRE AND EXPLOSION HAZARD

Flash Point: Not Known LEL: Not Known UEL: Not Known
Extinguishing Media: CO_2, Dry Chemical

Special Firefighting Procedures: Wear full protective equipment including self-contained breathing apparatus (eye, body, respiratory).

Unusual Fire Hazards: Combustion may result in the release of toxic/hazardous gases.

SECTION IV - REACTIVITY DATA

Instability: Stable
Hazardous Polymerization: None
Imcompatibility: Keep away from excess heat
Hazardous Decomposition: None
Decomposition Products: Hydrogen sulfide (thermal), sulfur dioxide (combustion).

SECTION V - SPILL, LEAK, AND DISPOSAL PROCEDURES

Spill - Assure personnel safety. Collect solids in a container and neutralize with sodium carbonate. Wash area with dilute hydrogen peroxide. Follow applicable state and federal disposal laws.

Disposal - Observe applicable local, state, and federal regulations.

SECTION VI - SPECIAL PROTECTION EQUIPMENT

Use only with adequate ventilation. Keep container tightly closed.

Chemical safety goggles. Full-face respirator with mask. Rubber-soled boots. Impervious gloves. Rubber apron. Mechanical hood.

SECTION VII - HEALTH HAZARD DATA

TLV: Not Known Mutagenicity: Not Known LD_{50}: Not Known

Effects of Overexposure
The vapors are irritating to the nose and the upper respiratory tract. Contact with skin, eyes, and mucous membranes should be always avoided. The toxicological properties of this compound have not been fully investigated. Regard this compound as a poison.

First Aid
In case of contact with skin rinse the affected area(s) with copious amounts of soap and water for at least 15 minutes. Seek medical advice. Launder clothing before reuse.

In case of contact with eyes, immediately flush with water for at least 15 minutes and seek medical attention.

Immediately move personnel to open air should excessive inhalation of vapors result in loss of consciousness or breathing difficulty. Provide necessary respiratory assistance. Seek medical attention quickly.

SECTION VIII - SPECIAL PRECAUTIONS

Store in cool, dry place in an air-tight container. Use in properly ventilated areas only.

The data provided is correct to the best of our knowledge. We shall not be held liable for any damage resulting from handling or from contact with above product. See pages 4-5, Catalog No. 8 for additional terms and conditions of sale.

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XI. GLP AUDIT SUMMARY

The 14-day range-finding study on 1,4-dithiane was exempt from GLP compliance because it was a preliminary range-finding study. Even though technically exempt from GLP compliance, the 14-day study had the following GLP audits and inspections:

Type of Audit or Inspection	Date Inspected	Date Reported	QA Inspector
Protocol Review	12/18/85	12/18/85	JB[a]
Review of Revised Protocol	1/9/86	1/9/86	JB
Diet Analysis,	1/12/86	3/24/86	AS[b]
Mixing, and	1/17/86	3/24/86	AS
Delivery Procedures	1/26/86	3/31/86	AS
Interim QA Review of Study	2/9-2/24/86	2/24/86	DT[c]
Final Report Review	1/9/86	3/13/87	DT

[a]John Berky, Ph.D., Director of QA at NCTR, now retired.

[b]Ammon Swartzentruber, a QA inspector at NCTR and Acting Director of QA.

[c]Delbert Taylor, a QA inspector at NCTR.

The 90-day subchronic toxicity study on 1,4-dithiane was conducted as a GLP experiment and had the following audits and GLP inspections:

Type of Audit or Inspection	Date Inspected	Date Reported	QA Inspector
Protocol Review	12/18/85	12/18/85	JB[a]
Review of Revised Protocol	1/9/86	1/9/86	JB
Start-up Meeting for Support Personnel	2/24/86	2/24/86	DT[b]
Animal Room Animal Allocation	4/2/86	4/2/86	AS[c]
Collection of Animal Weights and Observations	4/8-4/11/86	4/11/86	AS
Gavage Procedures	4/8-4/11/86	4/11/86	AS
Diet Analysis, Mixing, and Delivery Procedures	4/20/86	5/14/86	AS

Review of Protocol Support Procedures	4/27/86	4/27/86	DT
Review of Computer-Supported Animal Allocation	4/28/86	4/28/86	DT
Diet Analysis, Mixing, and Delivery Procedures	5/4/86	5/14/86	AS
Verification of Computer-Stored Data	5/16/86	5/29/86	DT
Review of Final Report	1/9/87	3/18/87	DT

[a]John Berky, Ph.D., Director of QA at NCTR, now retired.

[c]Delbert Taylor, a QA inspector at NCTR.

[b]Ammon Swartzentruber, a QA inspector at NCTR and Acting Director of QA.

Results of the Quality Assurance investigations and audits on Experiment 491 were reported to management on the dates indicated. All work observed was conducted in compliance with the Good Laboratory Practice Regulations promulgated by the Food and Drug Administration.

Ammon Swartzentruber
Acting Director, QA

The study was conducted in accordance with FDA Good Laboratory Practice Regulations and no problems were encountered which would adversely affect the scientific validity of the study.

George J. Schieferstein, Ph.D.
Principal Investigator

XII. DRINKING WATER CRITERION

A suggested drinking-water criterion for 1,4-dithiane for the protection of human health was calculated according to EPA guidelines (1979, 1980). Since the toxicity data on 1,4- dithiane are limited to 14- and 90-day data, the acceptable daily intake (ADI) was derived from the 90-day toxicity data.

The subchronic (90-day) study showed a significant ($p<.05$) increase in the female livers and in the male kidneys at the highest dose level (420 mg/kg/day). No change was found in organ weight in either sex at the lower dose levels of 105 or 210 mg/kg/day for 90 days. Hypertrophy of centrilobular hepatocytes was observed only in the females at the 420 mg/kg/day dose level. Anisotropic crystals of an undetermined chemical composition deposited in the nasal olfactory mucosa. This crystal deposition was seen in lowest dose level (105 mg/kg/day) in the males and in the females at an incidence of 6.7 and 82.8%, respectively. Based on these toxicologic data, a severity rating factor (RVe, see Table 31) of 3 was assigned at a dose level of 210 mg/kg/day.

Table 31. Rating Constants (RVe) for Noncarcinogens[1]

Effect	Severity Rating (RVe)
Enzyme induction or other biochemical change with no pathologic changes and no change in organ weights.	1
Enzyme induction and subcellular proliferation or other changes in organelles but no other apparent effects.	2
Hyperplasia, hypertrophy or atrophy, but no change in organ weights.	3
Hyperplasia, hypertrophy or atrophy with changes in organ weights.	4
Reversible cellular changes: cloudy swelling, hydropic change, or fatty changes.	5
Necrosis, or metaplasia with no apparent decrement of organ function. Any neuropathy without apparent behavioral, sensory, or physiologic changes.	6
Necrosis, atrophy, hypertrophy, or metaplasia with a detectable decrement of organ functions. Any neuropathy with a measurable change in behavioral, sensory, or physiologic activity.	7
Necrosis, atrophy, hypertrophy, or metaplasia with definitive organ dysfunction. Any neuropathy with gross changes in behavior, sensory, or motor performance. Any decrease in reproductive capacity, any evidence of fetotoxicity.	8
Pronounced pathologic changes with severe organ dysfunction. Any neuropathy with loss of behavioral or motor control or loss of sensory ability. Reproductive dysfunction. Any teratogenic effect with maternal toxicity.	9
Death or pronounced life-shortening. Any teratogenic effect without signs of maternal toxicity.	10

[1] Rating scale identical to that used by EPA in the [illegible] process, as described in EPA (1983).

An ADI was derived by estimating a no-observed-effect-level (NOEL) in the experimental animal and from this NOEL, a NOEL in humans. The first step in the process was to divide the lowest-observed-effect-level (LOEL) in the animal by the RVe as follows:

NOEL (animal) = LOEL/RVe.

The RVe was chosen according to EPA guidelines (EPA, 1986) given in Table 31. A human NOEL or ADI is obtained from the animal NOEL by dividing the appropriate uncertainty factor (10 for extrapolation from laboratory animals to humans, 10 for variability among humans, and 10 for extrapolation from subchronic to chronic exposure: an overall uncertainty factor of 1,000) as follows:

ADI = NOEL (human)
= NOEL (animal)/uncertainty factor.

Hence:

ADI = LOEL (animal)/(RVe x uncertainty factor).
= 210 mg/kg/day/(3 x 1,000).
= 0.07 mg/kg/day.

A suggested drinking water criterion (CW) is calculated from the ADI. In performing this calculation, a 70-kg adult human is assumed to drink 2 liters of water per day. Accordingly, the CW is obtained as follows:

CW = 0.07 mg/kg/day x 70 kg/2l/day.
= 2.45 *mg of 1,4-ditniane/l of water.*

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