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DETERMINATION OF THE TOXICITY OF CYCLOTRIPHOSPHAZENE HYDRAULIC FLUID BY 21-DAY REPEATED INHALATION AND DERMAL EXPOSURE

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TECHNICAL REVIEW AND APPROVAL

AAMRL-TR-89-028

The experiments reported herein were conducted according to the "Guide for the Care and Use of Laboratory Animals," Institute of Laboratory Animal Resources, National Research Council.

This report has been reviewed by the Office of Public Affairs (PA) and is releasable to the National Technical Information Service (NTIS). At NTIS, it will be available to the general public, including foreign nations.

This technical report has been reviewed and is approved for publication.

FOR THE COMMANDER

MICHAEL B. BALLINGER, Lt Col, USAF, BSC
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This study was designed to evaluate the toxic effects associated with repeated or continuous exposure to CTP by both the dermal and inhalation routes. Rats were exposed for three weeks to air alone, 0.25, 0.50 and 1.00 mg CTP/L. No deaths or signs of toxic stress occurred during the exposure period. A treatment-related depression in mean weight gain, increases in numbers of pulmonary alveolar macrophages and renal hyaline droplets was noted in both sexes. Rabbits were treated dermally for three weeks with mineral oil, or 0.25, and 1.00 g CTP/Kg. No toxic effects were noted in either sex of rabbits.

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11. Inhalation and Dermal Exposure.

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PREFACE

This is one of a series of technical reports describing results of the experimental laboratory programs conducted at the Toxic Hazards Research Unit, NSI Technology Services. This document serves as a final report on the in-life toxicity of cyclotriphosphazene hydraulic fluid The research described in this report began in July 1987 and was completed in October 1988 under U S. Air Force Contract No. F33615-85-C-0532. Melvin E. Andersen, Ph.D., served as Contract Technical Monitor for the U.S. Air Force, Harry G. Armstrong Aerospace Medical Research Laboratory. This study was sponsored by the U.S. Navy under the direction of CAPT David E. Uddin, MSC, USN, and CDR David A. Macys, MSC, USN.

This work was supported by the Naval Medical Research and Development Command Task MR04122010006. The opinions contained herein are those of the authors and are not to be construed as official or reflecting the view of the Department of the Navy or the Naval Services at large.

The animals used in this study were handled in accordance with the principles stated in the *Guide for the Care and Use of Laboratory Animals*, prepared by the Committee on Care and Uses of Laboratory Animals of the Institute of Laboratory Animal Resources, National Research Council, DHHA, National Institute of Health Publication #86-23, 1985. and the Animal Welfare Act of 1966, as amended.

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ABBREVIATIONS

AAMRL	Armstrong Aerospace Medical Research Laboratory
CTP	Cyclotriphosphazene
dL	Deciliter
F-344	Fischer 344 (rats)
fL	Femtoliter
g	Gram
GC	Gas chromatography
GSD	Geometric standard deviation
h	Hour
IU	International units
kg	Kilogram
L	Liter
µm	Micrometer
mg	Milligram
mm	Millimeter
MMAD	Mass median aerodynamic diameter
N	Number
NMRI/TD	Naval Medical Research Institute/Toxicology Detachment
NZW	New Zealand White (rabbits)
p	Probability
pg	Picogram
SD	Standard deviation
SEM	Standard error mean

SECTION 1

INTRODUCTION

The Navy has developed candidate hydraulic fluids with chemical structures of cyclotriphosphazene (CTP) cyclic esters. The hydraulic fluid of current interest contains 0.1% tolyltriazole, an additive that inhibits copper corrosion. Acute toxicity studies demonstrated that this hydraulic fluid is non-toxic by oral or dermal administration (Kinkead and Bowers, 1985; Kinkead and Bashe, 1987). Eye and skin irritation tests, as well as skin sensitization tests, proved negative (Kinkead and Bowers, 1985). The hydraulic fluid was not detected in the blood or urine of rats following exposure by aerosol inhalation or dermal contact (Kinkead and Bashe, 1987). The oral LD_{50} of tolyltriazole in rats is 675 mg/kg (HRCR, 1972)

The Toxicology Detachment of the Naval Medical Research Institute (NMRI/TD) requested that this laboratory study the effects of repeated exposures by both the dermal and inhalation routes. These experiments were designed to measure the toxic effects associated with repeated or continuous exposure to CTP over a limited time. An additional objective was to determine the "no observed effect" level of the compound. These studies were not designed to identify those effects which have a long latency period (e.g., carcinogenicity, decreased life expectancy).

SECTION 2

MATERIALS

TEST AGENT

The CTP cyclic ester hydraulic fluid, supplied by NMRI/TD, Wright-Patterson Air Force Base, OH, contains 0.1% tolyltriazole. The CTP supplied for these studies was a mixture of parent compound isomers including dimers, trimers, and tetramers of CTP with an approximate molecular weight of the fluids being 1000 g/mole. Other pertinent data on these materials are provided below:

Cyclotriphosphazene ester:	
NMRI/TD No.	87-174-01
CAS No.	291-37-2
Vapor Pressure, mmHg	65°C: 0.49 149°C: 12.0
Specific Gravity (g/mL)	1.445
Tolyltriazole:	
Chemical Formula	$C_7H_7N_3$
CAS No.	29385-43-1
Synonym	Methyl-1H-benzotriazole

TEST AGENT QUALITY CONTROL

A Varian 3700 gas chromatograph (GC) equipped with a flame ionization detector and a 50-meter, 5% phenylmethyl silicone coated, fused silica capillary column was utilized in conjunction with a Hewlett-Packard 3388 computing integrator to measure peak area and record chromatograms of the test material. Profiles were obtained of the material as received, as aerosolized, and as residue from the nebulizer system.

ANIMALS

Male and female Fischer 344 (F-344) rats, 9 to 11 weeks of age at the onset of the inhalation study, were purchased from Charles River Breeding Labs, Kingston, NY. Upon receipt, the animals were weighed and, beginning with animals at one extreme of the weight range, they were randomly assigned to the four experimental groups. All rats were judged to be in good health following a two-week quarantine period. Prior to the study, rats were group-housed (two to three per cage) in clear plastic cages with wood chip bedding. During the study, the rats were continuously housed in individual cages within the exposure chambers. Each exposure day, the cages were rotated one position in a clockwise direction within the chambers. Water and feed (Purina Formulab #5008) were available *ad libitum* except that food was withheld during the exposures and for 10 h prior to sacrifice. Rats were maintained on a 12-h light/dark cycle.

Male and female New Zealand White (NZW) rabbits, 2 to 3 kg in weight, were obtained from Clerco Research Farms (Cincinnati, OH) for use in the dermal studies. Quality control evaluations confirmed the satisfactory health of the study animals. The rabbits were randomized into treatment groups in the same manner as that described for rats. The rabbits were housed individually in wire-bottom stainless-steel cages. Water and food (Purina Rabbit Chow #5320 and/or MannaPro Rabbit Family Ration) were available *ad libitum*. Rabbits were maintained on a 12-h light/dark cycle.

INHALATION TOXICITY STUDY

Aerosol exposure atmospheres were generated using a one, three, or six-jet Collison compressed-air nebulizer (BGI, Inc., Waltham, MA) depending on the concentration of CTP required. The aerosol concentration within the exposure chamber was measured hourly by gravimetric analysis of droplets collected on a glass-fiber filter media. Vapor concentrations of CTP were assessed by bubbling filtered chamber atmosphere through isopropanol to trap the test agent for GC analysis. A Lovelace Multijet Cascade Impactor (Intox Products, Albuquerque, NM) was used to assess the size distribution of aerosol in each chamber. Impactor samples were analyzed by GC to determine the stability and overall composition of the aerosol. All aerosol exposures were carried out in 690 L Hinners (Hinners et al., 1968) type inhalation chambers. Air flow through the chambers was maintained at 12 to 15 chamber volumes per h.

Ten male and 10 female F-344 rats were placed in each of four inhalation chambers and exposed to either 0.00-, 0.25-, 0.50-, or 1.00-mg CTP/L for 6 h/day, 5 days/week, for three weeks (i.e., 15 exposures over a 21-day test period). Records were maintained for body weight (Days 0, 7, 14, and 21), signs of toxicity, and mortality. At sacrifice, gross pathological findings were noted, blood was drawn for hematology and clinical chemistry assays (Table 1), and tissues were harvested for histopathologic examination (Table 2). Wet tissue weights were recorded for adrenal glands, brain, heart, kidneys, liver, lungs, ovaries (females), spleen, testes (males), and thymus.

TABLE 1. HEMATOLOGY AND CLINICAL CHEMISTRY PARAMETERS ASSESSED IN F-344 RATS AND NZW RABBITS FOLLOWING TREATMENT WITH CTP

Hematology	Chemistry
Hematocrit	Creatinine
Hemoglobin	Lactate dehydrogenase
Red blood cell count	Calcium
Total leucocyte count	Phosphorus
Differential leucocyte count	Total protein
	Alkaline phosphatase
	Blood urea nitrogen
	Serum glutamic-pyruvic transaminase
	Serum glutamic-oxaloacetic transaminase

TABLE 2. TISSUES HARVESTED FOR HISTOPATHOLOGIC EXAMINATION FOLLOWING EXPOSURE OF F-344 RATS TO CTP

Gross lesions	Thymus
Thyroid/parathyroid	Brain
Lungs	Kidneys
Trachea	Adrenal glands
Heart	Pancreas
Liver	Gonads
Spleen	Nasal turbinates (3 sections)
Duodenum	Uterus (females)
Jejunum	Esophagus
Ileum	Stomach
Urinary bladder	Colon
Mandibular lymph nodes	Rectum
Mesenteric lymph nodes	Sternum
Eye	Sciatic nerve
Preputial glands	Skeletal muscle
Pituitary glands	

DERMAL TOXICITY STUDY

Four groups of 10 male and 10 female NZW rabbits were used in the dermal toxicity studies. Hair was clipped from the rabbits' backs prior to the first treatment and twice a week thereafter throughout the study. Each animal within a group was treated on weekdays only, for three weeks, with either 1.00 g mineral oil/kg (control), or 0.25-, 0.50-, or 1.00-g CTP/kg. The appropriate dose volumes were adjusted using the weekly individual animal body weights.

Test and control materials were applied directly to the skin and covered with 4-ply gauze squares. The gauze was covered by a layer of clear plastic wrap (Glad Cling Wrap, First Brands Corporation, Danbury, CT) around the entire rabbit midsection and secured with an elastic bandage (Vetrap, 3M Corporation, Minneapolis, MN). After each 6-h treatment the tape, plastic wrap, and gauze were removed and any residual material was wiped from the skin.

Body weights were measured immediately prior to the first treatment (Day 0), and on Days 7, 14, and 21. Blood was drawn from each of five animals of each sex of each treatment group one day prior to the first dose and at sacrifice for hematology and clinical chemistry assays (Table 1). The animals were observed daily and any signs of toxicity were recorded. Test and control groups were sacrificed on the day following the 15th treatment. During necropsy, gross pathological lesions were noted, blood was drawn, and tissues (Table 3) were harvested for histopathologic examination. Wet

tissue weights were obtained at sacrifice for adrenal glands, brain, heart, kidneys, liver, lungs, ovaries (female), spleen, testes (male), and thymus.

TABLE 3. TISSUES HARVESTED FOR HISTOPATHOLOGIC EXAMINATION FOLLOWING TREATMENT OF NZW RABBITS WITH CTP

Gross lesions	Thymus
Normal and treated skin	Brain
Lungs	Kidneys
Trachea	Adrenal glands
Heart	Pancreas
Liver	Gonads
Spleen	Uterus (females)
Duodenum	Esophagus
Jejunum	Stomach
Ileum	Cecum
Urinary bladder	Colon
Mandibular lymph nodes	Rectum
Mesenteric lymph nodes	Sternum
Gallbladder	Sciatic nerve
Pituitary	Thyroid/parathyroid
Skeletal muscle	Eye

STATISTICAL ANALYSIS

Mean group body weights were compared using the Multivariate Analysis of Covariance for Repeated Measures Test (Barcikowski, 1983; Dixon, 1985). A two-factorial analysis of variance with multivariate comparisons was applied to the hematology, clinical chemistry, and organ weight data. Histopathological data were analyzed using the following nonparametric tests: Fisher's Exact Test and Yates' Corrected Chi-square (Zar, 1974). A probability of <0.05 was considered a statistically significant change from controls.

SECTION 3

RESULTS

INHALATION TOXICITY STUDY

Chamber Analysis

GC analysis of aerosolized CTP from the chambers, test material prior to aerosolization, and residual CTP remaining in the generators indicated that the composition of the test material did not change during the course of the 6-h exposure periods. CTP vapor was not detected in the chamber atmospheres.

During the three-week exposure period, daily mean concentrations of CTP were maintained within 10% of the desired concentration, except on the 13th exposure day when the mean daily concentration of the 0.25 mg/L chamber was 84% of nominal (Table 4). Examination of the distribution of aerosol size in each exposure chamber indicated that the droplets were of respirable size (Table 5).

TABLE 4. TIME WEIGHTED AVERAGE (± SD) CONCENTRATIONS OF CTP IN ANIMAL EXPOSURE CHAMBERS

	Target Concentrations		
Study Day	0.25 mg/L	0.50 mg/L	1.00 mg/L
1[a]	0.25 ± 0.02	0.49 ± 0.03	1.02 ± 0.09
2	0.24 ± 0.03	0.50 ± 0.03	1.01 ± 0.06
3	0.25 ± 0.04	0.51 ± 0.03	1.01 ± 0.04
4	0.25 ± 0.03	0.51 ± 0.02	0.96 ± 0.03
5	0.26 ± 0.03	0.50 ± 0.01	0.98 ± 0.03
6	0.25 ± 0.03	0.49 ± 0.02	0.98 ± 0.12
7	0.23 ± 0.04	0.52 ± 0.05	0.98 ± 0.05
8	0.24 ± 0.02	0.52 ± 0.01	1.00 ± 0.02
9	0.25 ± 0.02	0.51 ± 0.01	1.01 ± 0.05
10	0.26 ± 0.01	0.51 ± 0.03	0.98 ± 0.05
11	0.25 ± 0.02	0.50 ± 0.03	1.00 ± 0.15
12	0.23 ± 0.01	0.51 ± 0.03	0.99 ± 0.06
13	0.21 ± 0.06	0.50 ± 0.03	0.99 ± 0.11
14	0.24 ± 0.05	0.51 ± 0.04	1.01 ± 0.06
15	0.25 ± 0.02	0.52 ± 0.03	0.98 ± 0.08
16[b]	0.25 ± 0.01	0.51 ± 0.02	1.00 ± 0.04
Mean ± SD	**0.24 ± 0.01**	**0.51 ± 0.01**	**0.99 ± 0.02**

[a] Male rats only
[b] Female rats only

TABLE 5. PARTICLE SIZE OF AEROSOLS IN CTP INHALATION EXPOSURES

Target Conc. (mg/L)	MMAD (μm)[a]	GSD (range)[b]
0.25	2.18 ± 0.04	1.67 – 2.33
0.50	1.96 ± 0.01	1.68 – 2.18
1.00	2.22 ± 0.01	1.80 – 2.32

[a] Mass median aerodynamic diameter, ± S.E.M. (N = 32)
[b] Geometric standard deviation range observed for individual MMAD values

Biological Data

Because exposures of female rats were begun one day after the male rats, the total calendar days for the study numbers 16; however, each group received only 15 exposures (Table 4). A total of 80 F-344 rats were included in the three-week inhalation toxicity study. There were no behavioral or physical signs of toxic stress observed during the exposure period and no deaths occurred. Despite the random assignments to exposure groups, there were significant differences between the mean group weights of male rats at the start of the exposures (Table 6 and Appendix 1). Therefore, mean weight gains and losses were analyzed. Weight gains of treatment groups were significantly depressed when compared to the respective control group of the same sex after one week of exposure. However, after the first week, weight changes were similar among groups of the same sex.

TABLE 6. MEAN BODY WEIGHTS[a] (g) OF F-344 RATS DURING 21-DAY INHALATION EXPOSURE TO CTP

Sex	Dose Group	Day 0	Day 7	Day 14	Day 21[b]
Male	Control	199 ± 6	226 ± 2	240 ± 2	234 ± 2
	0.25 mg/L	194 ± 5[c]	213 ± 2[d,f]	228 ± 3[d]	222 ± 3[d]
	0.50 mg/L	210 ± 3[d]	215 ± 3[d,f]	229 ± 3[d]	223 ± 3[d]
	1.00 mg/L	199 ± 4	207 ± 3[d,f]	223 ± 3[d]	218 ± 3[d]
Female	Control	147 ± 2	151 ± 2	157 ± 2	147 ± 2
	0.25 mg/L	151 ± 1	148 ± 2[e]	153 ± 1	146 ± 2
	0.50 mg/L	147 ± 2	144 ± 2[c,e]	148 ± 3[d]	143 ± 3
	1.00 mg/L	148 ± 3	145 ± 3[c,e]	151 ± 2[c]	146 ± 3

[a] Mean ± S E M , N = 10

[b] Fasted weights

[c] Significantly different from control at $p < 05$ using Multivariate Analysis of Covariance for Repeated Measures Test

[d] Significantly different from control at $p < 01$ using Multivariate Analysis of Covariance for Repeated Measures Test

[e] Seven day weight gain significantly less than controls at $p < 05$ using Multivariate Analysis of Covariance for Repeated Measures Test

[f] Seven day weight gain significantly less than controls at $p < 01$ using Multivariate Analysis of Covariance for Repeated Measures Test

None of the clinical chemistry parameters revealed any significant effects on the exposed rats (Tables 7, 8). Analysis of hematology parameters (Tables 9, 10) revealed no differences between test and control groups, and all group means for these parameters were within the normal range for the age and species of test animals used (Wolford et al , 1986).

TABLE 7. MEAN VALUES[a] OF SERUM CHEMISTRY PARAMETERS FOR MALE F-344 RATS FOLLOWING 21-DAY REPEATED INHALATION EXPOSURE TO CTP

Parameter	Control	0.25 mg/L	0.50 mg/L	1.00 mg/L
BUN (mg/dL)	16.9 ± 0.5	17.9 ± 0.7	16.5 ± 0.4	16.9 ± 0.6
Creatinine (mg/dL)	0.6 ± <0.1	0.6 ± <0.1	0.6 ± <0.1	0.6 ± <0.1
Calcium (mg/dL)	10.6 ± 0.2	10.7 ± 0.1	10.7 ± 0.2	10.7 ± 0.2
Total protein (g/dL)	7.5 ± 0.1[b]	7.3 ± 0.1[b]	7.4 ± 0.1[c]	7.5 ± 0.1[b]
Alk. phos. (IU/L)	185.7 ± 10.2	182.1 ± 8.7	174.7 ± 5.3	177.1 ± 5.6
LDH (IU/L)	625.3 ± 99.3	712.5 ± 95.4	596.9 ± 52.7	826.8 ± 110.4

[a] Mean ± S.E.M., N = 10 except where noted

[b] N = 7

[c] N = 6

TABLE 8. MEAN VALUES[a] OF SERUM CHEMISTRY PARAMETERS FOR FEMALE F-344 RATS FOLLOWING 21-DAY REPEATED INHALATION EXPOSURE TO CTP

Parameter	Control	0.25 mg/L	0.50 mg/L	1.00 mg/L
BUN (mg/dL)	21.7 ± 0.7	21.1 ± 0.7	22.5 ± 1.2	22.3 ± 0.7[b]
Creatinine (mg/dL)	0.6 ± <0.1	0.6 ± <0.1	0.6 ± <0.1	0.6 ± <0.1[b]
Calcium (mg/dL)	10.7 ± 0.2	10.1 ± 0.2	10.3 ± 0.2	9.9 ± 0.2[c]
Total protein (g/dL)	7.1 ± 0.1	7.0 ± <0.1	6.9 ± 0.3	7.1 ± 0.1[c]
Alk. phos. (IU/L)	124.1 ± 4.1	128.7 ± 9.0	125.8 ± 9.2	125.5 ± 3.5[c]
LDH (IU/L)	584.0 ± 125.3	745.6 ± 135.5	465.3 ± 90.7	783.1 ± 149.8[b]

[a] Mean ± S.E.M., N = 10 except where noted

[b] N = 9

[c] N = 8

TABLE 9. MEAN[a] WHOLE BLOOD PARAMETERS FOR MALE F-344 RATS FOLLOWING 21-DAY INHALATION EXPOSURE TO CTP

Parameter	Control	0.25 mg/L	0.50 mg/L	1.00 mg/L
WBC ($\times 10^3$ cells/mm^3)	8.00 ± 0.51	7.66 ± 0.35	7.60 ± 0.34	7.16 ± 0.53
RBC ($\times 10^6$ cells/mm^3)	8.56 ± 0.14	8.46 ± 0.09	8.63 ± 0.07	8.18 ± 0.12
HGB (g/dL)	16.99 ± 0.19	16.63 ± 0.10	16.71 ± 0.12	16.22 ± 0.23
HCT (%)	45.48 ± 0.75	44.53 ± 0.60	45.31 ± 0.46	42.88 ± 0.58
MCV (fL)	52.99 ± 0.34	52.52 ± 0.41	52.48 ± 0.25	52.44 ± 0.31
MCH (pg)	19.89 ± 0.17	19.68 ± 0.17	19.39 ± 0.15	19.85 ± 0.15
MCHC (%)	37.50 ± 0.27	37.45 ± 0.39	36.90 ± 0.23	38.02 ± 0.31
Neutrophils (%)	27.30 ± 2.68	20.40 ± 1.82	22.40 ± 1.66	25.50 ± 1.71
Lymphocytes (%)	68.10 ± 2.88	77.10 ± 1.59	75.20 ± 2.00	70.80 ± 1.70
Monocytes (%)	2.38 ± 0.46	2.00 ± 0.70	2.33 ± 0.33	2.17 ± 0.40
Eosinophils (%)	1.20 ± 0.20	1.17 ± 0.17	1.17 ± 0.17	2.00 ± 0.58
Atypical Lymphocytes (%)	2.25 ± 0.31	1.25 ± 0.16	1.60 ± 0.60	1.88 ± 0.40

[a] Mean ± S.E.M., N = 10

TABLE 10. MEAN[a] WHOLE BLOOD PARAMETERS FOR FEMALE F-344 RATS FOLLOWING 21-DAY INHALATION EXPOSURE TO CTP

Parameter	Control[b]	0.25 mg/L	0.50 mg/L[c]	1.00 mg/L
WBC ($\times 10^3$ cells/mm^3)	9.72 ± 0.93	10.94 ± 1.22	11.36 ± 1.19	10.77 ± 0.68
RBC ($\times 10^6$ cells/mm^3)	7.87 ± 0.15	7.72 ± 0.11	7.63 ± 0.10	7.64 ± 0.09
HGB (g/dL)	16.40 ± 0.30	15.85 ± 0.20	15.74 ± 0.17	15.82 ± 0.17
HCT (%)	42.72 ± 0.88	41.74 ± 0.60	41.44 ± 0.48	41.35 ± 0.51
MCV (fL)	54.19 ± 0.28	54.14 ± 0.37	54.23 ± 0.41	54.07 ± 0.21
MCH (pg)	20.86 ± 0.14	20.55 ± 0.22	20.65 ± 0.17	20.53 ± 0.22
MCHC (%)	44.00 ± 5.69	38.26 ± 0.20	38.00 ± 0.35	38.03 ± 0.34
Neutrophils (%)	19.78 ± 1.98	21.00 ± 1.69	20.63 ± 2.58	21.30 ± 2.45
Lymphocytes (%)	78.00 ± 2.18	75.20 ± 1.95	76.50 ± 2.75	74.20 ± 2.33
Monocytes (%)	1.38 ± 0.18	3.00 ± 0.91	2.00 ± 0.31	2.75 ± 0.73
Eosinophils (%)	1.00 ± < 0.00	1.71 ± 0.47	1.00 ± < 0.01	2.11 ± 0.20
Atypical lymphocytes (%)	2.50 ± 0.50	2.50 ± 0.65	0.00 ± < 0.01	1.00 ± < 0.01

[a] Mean ± S.E.M., N = 10 except where noted

[b] N = 9

[c] N = 8

Organ weights and organ to body weight ratios, measured at necropsy (Tables 11, 12), identified the spleen, liver, and testes of male exposed rats and the liver of female exposed rats as different from those of their respective controls. Decreases in absolute spleen weights in the 0.25 and 1.0 mg CTP/L groups were not confirmed by comparisons of spleen to body weight ratios. Although the liver to body weight ratios of all test groups of both sexes appeared slightly greater than their respective controls, the increases were significant only for the 0.5 mg CTP/L male rats and the 1.0 mg CTP/L female rats. Testes to body weight ratios were significantly ($p<0.01$) greater than controls for all three exposure groups.

TABLE 11. MEAN ORGAN WEIGHTS[a] (g) AND ORGAN TO BODY WEIGHT RATIOS (%) OF MALE F-344 RATS FOLLOWING 21-DAY INHALATION EXPOSURE TO CTP

Parameter	Control	0.25 mg/L	0.50 mg/L	1.00 mg/L
Adrenal glands	0.07 ± 0.01	0.09 ± 0.02	0.07 ± 0.01	0.06 ± < 0.01
Ratio[b]	0.03 ± < 0.01	0.04 ± 0.01	0.03 ± < 0.01	0.03 ± < 0.01
Brain	1.73 ± 0.03	1.76 ± 0.03	1.98 ± 0.17	1.73 ± 0.03
Ratio	0.74 ± 0.01	0.79 ± 0.02	0.89 ± 0.08	0.80 ± 0.01
Heart	0.82 ± 0.02	0.82 ± 0.03	0.80 ± 0.02	0.79 ± 0.02
Ratio	0.35 ± 0.01	0.37 ± 0.01	0.36 ± 0.01	0.36 ± 0.01
Kidney	1.84 ± 0.03	1.77 ± 0.04	1.80 ± 0.04	1.76 ± 0.04
Ratio	0.79 ± 0.01	0.80 ± 0.01	0.81 ± 0.01	0.81 ± 0.01
Liver	7.83 ± 0.17	7.53 ± 0.19	7.79 ± 0.19	7.30 ± 0.52
Ratio	3.34 ± 0.06	3.39 ± 0.05	3.50 ± 0.05[c]	3.35 ± 0.23
Lung	1.62 ± 0.07	1.44 ± 0.04	1.72 ± 0.18	1.54 ± 0.05
Ratio	0.69 ± 0.03	0.65 ± 0.02	0.77 ± 0.08	0.71 ± 0.02
Testes	2.95 ± 0.03	2.97 ± 0.01	2.93 ± 0.05	2.92 ± 0.04
Ratio	1.26 ± 0.01	1.34 ± 0.02[d]	1.32 ± 0.02[d]	1.34 ± 0.01[d]
Spleen	0.54 ± 0.02	0.48 ± 0.01[c]	0.51 ± 0.02	0.47 ± 0.01[d]
Ratio	0.23 ± 0.01	0.22 ± 0.01	0.23 ± 0.01	0.22 ± 0.01
Thymus	0.32 ± 0.02	0.29 ± 0.03	0.33 ± 0.02	0.30 ± 0.02
Ratio	0.14 ± 0.01	0.13 ± 0.01	0.15 ± 0.01	0.14 ± 0.01
Whole body (g)	**234.10 ± 2.25**	**222.10 ± 2.74[d]**	**222.70 ± 3.10[d]**	**217.80 ± 2.54[d]**

[a] Mean ± S E M , N = 10
[b] Organ weight/body weight × 100
[c] Significantly different from controls at p< 05 using a two-factorial analysis of variance with multivariate comparisons
[d] Significantly different from controls at p< 01 using a two-factorial analysis of variance with multivariate comparisons

TABLE 12. MEAN ORGAN WEIGHTS[a] (g) AND ORGAN TO BODY WEIGHT RATIOS (%) OF FEMALE F-344 RATS FOLLOWING 21-DAY INHALATION EXPOSURE TO CTP

Parameter	Control	0.25 mg/L	0.50 mg/L	1.00 mg/L
Adrenal glands	0.07 ± 0.01	0.07 ± < 0.01	0.07 ± 0.01	0.07 ± < 0.01
Ratio[b]	0.05 ± 0.01	0.05 ± < 0.01	0.05 ± 0.01	0.05 ± < 0.01
Brain	1.72 ± 0.02	1.74 ± 0.02	1.69 ± 0.03	1.71 ± 0.03
Ratio	1.17 ± 0.02	1.19 ± 0.01	1.18 ± 0.03	1.18 ± 0.03
Heart	0.59 ± 0.01	0.59 ± 0.02	0.58 ± 0.01	0.58 ± 0.01
Ratio	0.40 ± 0.01	0.40 ± 0.01	0.40 ± 0.01	0.40 ± 0.01
Kidney	1.17 ± 0.02	1.20 ± 0.02	1.19 ± 0.03	1.17 ± 0.03
Ratio	0.79 ± 0.01	0.82 ± 0.01	0.83 ± 0.02	0.80 ± 0.02
Liver	4.31 ± 0.10	4.69 ± 0.10	4.73 ± 0.11	4.90 ± 0.09
Ratio	2.93 ± 0.03	3.21 ± 0.04	3.30 ± 0.06	3.38 ± 0.06[c]
Lung	1.16 ± 0.02	1.18 ± 0.03	1.16 ± 0.02	1.29 ± 0.03
Ratio	0.79 ± 0.01	0.81 ± 0.02	0.81 ± 0.02	0.89 ± 0.03
Ovaries	0.11 ± 0.01	0.13 ± 0.01	0.12 ± < 0.01	0.15 ± 0.02
Ratio	0.08 ± 0.01	0.09 ± 0.01	0.09 ± < 0.01	0.10 ± 0.01
Spleen	0.40 ± 0.01	0.41 ± 0.02	0.38 ± 0.01	0.41 ± 0.01
Ratio	0.27 ± < 0.01	0.28 ± 0.01	0.27 ± 0.01	0.28 ± 0.01
Thymus	0.30 ± 0.02	0.30 ± 0.02	0.28 ± 0.01	0.30 ± 0.01
Ratio	0.21 ± 0.01	0.21 ± 0.01	0.19 ± 0.01	0.21 ± 0.01
Whole body (g)	**146.80 ± 2.19**	**146.20 ± 1.47**	**143.30 ± 2.51**	**145.50 ± 2.62**

[a] Mean ± S E M , N = 10
[b] Organ weight/body weight × 100
[c] Significantly different from controls at p< 05 using a two factorial analysis of variance with multivariate comparisons

Gross pathological examination of the animals at necropsy failed to reveal any CTP-related lesions. Light microscopy revealed excess numbers of pulmonary alveolar macrophages (Table 13) in 100% of the 1.00 mg/L groups. The percentage of responding animals decreased in the mid- and low-concentration groups, respectively, to 50% or fewer control animals exhibiting alveolar macrophages (alveolar histiocytosis). The severity of alveolar histiocytosis was significant ($p<0.01$) in the highest concentration male and female rats (Table 13).

Hyaline droplets were seen in the kidneys of CTP exposed animals (100%, 100%, and 50% for males and 80%, 80% and 70% for females exposed to 1.0-, 0.5-, and 0.25-mg CTP/L, respectively), while none were seen in the kidneys of control animals. The severity of the hyaline droplet lesions was significant in all CTP exposure groups (Table 13).

TABLE 13. SUMMARY OF SELECTED MICROSCOPIC LESIONS OBSERVED IN F-344 RATS FOLLOWING 21-DAY INHALATION EXPOSURE TO CTP

	Incidence (%)				Severity[a]			
Organ – Lesion	Control	0.25 mg/L	0.50 mg/L	1.00 mg/L	Control	0.25 mg/L	0.50 mg/L	1.00 mg/L
Lung – Alveolar macrophages								
Male	20	20	70	100[b]	0.2	0.2	0.7	1.1[b]
Female	50	60	60	100[b]	0.5	0.6	0.6	1.1[b]
Kidney – *Hyaline droplets*								
Male	0	50[b]	100[b]	100[b]	0.0	0.5[c]	1.0[b]	1.0[b]
Female	0	70[b]	80[b]	80[b]	0.0	0.7[b]	0.8[b]	0.8[b]

[a] Severity scoring system defined as: 0 = no lesion, 1 = minor or very slight, 2 = slight, 3 = moderate; 4 = marked, 5 = severe. Group scores are calculated by dividing the sum of individual scores by the number of affected animals.
[b] Significantly different from control, $p<0.01$ using Fisher's Exact Test and Yates' Corrected Chi-Square Test.
[c] Significantly different from control, $p<0.05$ using Fisher's Exact Test and Yates' Corrected Chi-Square Test.

DERMAL TOXICITY STUDY

A total of 80 NZW rabbits were used in the three-week dermal toxicity study. Two male rabbits were euthanatized, following accidental injury, during the course of the study; one rabbit from the 1.00g/kg group, and the second from the 0.25 g/kg group. Neither behavioral abnormalities nor signs of toxic stress were observed in the study animals at any time during the three-week treatment regimen. All groups gained weight during the course of the study (Table 14 and Appendix 2). Statistical analysis of body weights confirmed that any observed differences in mean group weights were not treatment-related.

TABLE 14. MEAN BODY WEIGHTS[a] (kg) OF NZW RABBITS DURING 21-DAY REPEATED DERMAL TREATMENT WITH CTP

Sex	Dose Group	Body Weights (kg)			
		Day 0	Day 7	Day 14	Day 21
Male	Control	2.7 ± 0.1	2.8 ± 0.1	2.9 ± 0.1	3.0 ± 0.1
	0.25 g/kg	2.8 ± 0.1	2.9 ± 0.1	3.0 ± 0.1	3.1 ± 0.1[b]
	0.50 g/kg	2.7 ± 0.1	2.8 ± 0.1	2.9 ± 0.1	3.0 ± 0.1
	1.00 g/kg	2.8 ± 0.1	2.9 ± 0.1[b]	3.0 ± 0.1[b]	3.0 ± 0.1[b]
Female	Control	2.7 ± < 0.1	2.8 ± 0.1	2.9 ± 0.1	3.0 ± 0.1
	0.25 g/kg	2.9 ± < 0.1	2.9 ± 0.1	3.1 ± 0.1	3.3 ± 0.1
	0.50 g/kg	2.8 ± 0.1	2.9 ± 0.1	3.0 ± 0.1	3.1 ± 0.1
	1.00 g/kg	2.8 ± 0.1	2.8 ± 0.1	2.8 ± 0.1	3.0 ± 0.1

[a] Mean ± S.E.M., N = 10 except where noted

[b] N = 9

Analysis of hematology data (Tables 15, 16) revealed no apparent exposure-related effects and all group means for these parameters were within the normal range for the age and species of these animals (Wolford et al., 1986). Clinical chemistry data (Tables 17, 18) indicated no significant differences from controls for any of the parameters evaluated.

TABLE 15. MEAN[a] WHOLE BLOOD PARAMETERS FOR NZW MALE RABBITS FOLLOWING 21-DAY REPEATED TREATMENT WITH CTP

Parameter	Control	0.25 mg/L	0.50 mg/L	1.00 mg/L
WBC (x 10^3 cells/mm^3)				
Pre-exposure	6.26 ± 0.64	6.48 ± 0.37	5.54 ± 0.35	6.14 ± 0.50
Postexposure	7.38 ± 0.99	6.30 ± 0.17	5.72 ± 0.43	6.26 ± 0.74
RBC (x 10^6 cells/mm^3)				
Pre-exposure	5.95 ± 0.15	5.53 ± 0.16	5.73 ± 0.17	5.62 ± 0.08
Postexposure	6.11 ± 0.07	5.77 ± 0.16	5.88 ± 0.17	5.84 ± 0.09
HGB (g/dL)				
Pre-exposure	12.88 ± 0.24	12.14 ± 0.24	12.50 ± 0.29	12.30 ± 0.16
Postexposure	13.44 ± 0.23	13.20 ± 0.13	13.04 ± 0.40	12.80 ± 0.09
HCT (%)				
Pre-exposure	37.52 ± 0.81	35.46 ± 0.88	36.16 ± 0.84	35.90 ± 0.47
Postexposure	38.60 ± 0.68	37.92 ± 0.33	37.46 ± 1.21	37.16 ± 0.36
MCV (fL)				
Pre-exposure	63.02 ± 0.53	64.14 ± 0.91	63.20 ± 1.05	64.10 ± 0.74
Postexposure	63.04 ± 0.91	64.34 ± 0.91	63.68 ± 0.99	63.60 ± 0.59
MCH (pg)				
Pre-exposure	21.62 ± 0.29	21.96 ± 0.29	21.82 ± 0.29	21.86 ± 0.21
Postexposure	21.96 ± 0.29	22.46 ± 0.35	22.20 ± 0.30	21.94 ± 0.25
MCHC (%)				
Pre-exposure	34.28 ± 0.24	34.26 ± 0.28	34.56 ± 0.26	34.26 ± 0.23
Postexposure	34.76 ± 0.13	34.88 ± 0.44	34.84 ± 0.29	34.44 ± 0.25
Neutrophils (%)				
Pre-exposure	41.60 ± 4.37	48.40 ± 4.01	52.20 ± 2.42	52.20 ± 1.46
Postexposure	48.60 ± 4.91	51.20 ± 6.72	46.80 ± 5.34	52.00 ± 2.84
Lymphocytes (%)				
Pre-exposure	55.00 ± 3.96	47.60 ± 4.81	45.40 ± 2.68	46.60 ± 1.21
Postexposure	48.80 ± 4.87	51.20 ± 6.72	52.00 ± 5.06	46.00 ± 3.15
Monocytes (%)				
Pre-exposure	4.33 ± 1.20	4.25 ± 0.75	1.80 ± 0.37	1.50 ± 0.50
Postexposure	3.67 ± 1.76	5.75 ± 0.63	2.00 ± 0.58	3.33 ± 0.33
Eosinophils (%)				
Pre-exposure	1.00 ± < 0.01	1.50 ± 0.50	1.50 ± 0.50	0.00 ± 0.00
Postexposure	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00

[a] Mean ± S.E.M. N = 5

TABLE 16. MEAN[a] WHOLE BLOOD PARAMETERS FOR NZW FEMALE RABBITS FOLLOWING 21-DAY REPEATED TREATMENT WITH CTP

Parameter	Control	0.25 mg/L	0.50 mg/L	1.00 mg/L
WBC ($\times 10^3$ cells/mm^3)				
Pre-exposure	7.20 ± 1.00	5.58 ± 0.59	6.78 ± 0.76	6.08 ± 0.51
Postexposure	7.78 ± 1.02	6.32 ± 0.88	7.02 ± 0.63	5.84 ± 0.51
RBC ($\times 10^6$ cells/mm^3)				
Pre-exposure	6.06 ± 0.27	5.72 ± 0.12	5.50 ± 0.20	5.70 ± 0.07
Postexposure	6.23 ± 0.23	5.69 ± 0.14	5.18 ± 0.16	5.73 ± 0.17
HGB (g/dL)				
Pre-exposure	13.08 ± 0.29	12.53 ± 0.34	12.34 ± 0.27	12.42 ± 0.14
Postexposure	13.40 ± 0.46	12.50 ± 0.22	12.08 ± 0.31	12.80 ± 0.25
HCT (%)				
Pre-exposure	39.65 ± 1.17	37.28 ± 0.93	36.24 ± 0.95	37.22 ± 0.25
Postexposure	39.82 ± 1.23	36.76 ± 0.69	33.58 ± 1.44	37.30 ± 0.69
MCV (fL)				
Pre-exposure	65.48 ± 1.35	65.13 ± 1.13	64.48 ± 1.19	65.20 ± 1.07
Postexposure	63.96 ± 1.32	64.54 ± 0.76	64.66 ± 1.25	65.12 ± 1.22
MCH (pg)				
Pre-exposure	21.68 ± 0.58	21.95 ± 0.37	22.00 ± 0.55	21.80 ± 0.37
Postexposure	21.56 ± 0.57	21.98 ± 0.42	23.44 ± 0.98	24.40 ± 2.15
MCHC (%)				
Pre-exposure	32.98 ± 0.38	33.45 ± 0.21	34.06 ± 0.31	33.28 ± 0.29
Postexposure	33.66 ± 0.21	34.04 ± 0.56	36.28 ± 1.68	34.38 ± 0.17
Neutrophils (%)				
Pre-exposure	37.00 ± 5.73	56.25 ± 7.76	38.40 ± 3.59	57.00 ± 2.30
Postexposure	50.20 ± 6.94	43.00 ± 6.91	38.20 ± 3.80	38.80 ± 5.38
Lymphocytes (%)				
Pre-exposure	61.75 ± 5.85	51.50 ± 8.73	38.40 ± 3.59	41.80 ± 2.71
Postexposure	47.00 ± 7.11	54.80 ± 6.77	61.00 ± 3.81	58.80 ± 4.88
Monocytes (%)				
Pre-exposure	1.25 ± 0.25	1.00 ± < 0.01	1.75 ± 0.48	1.25 ± 0.25
Postexposure	2.40 ± 0.68	1.80 ± 0.58	1.33 ± 0.33	2.20 ± 0.58
Eosinophils (%)				
Pre-exposure	0.00 ± 0.00	1.00 ± < 0.01	0.00 ± 0.00	1.00 ± < 0.01
Postexposure	1.00 ± < 0.01	1.00 ± < 0.01	0.00 ± 0.00	1.00 ± < 0.01

[a] Mean ± S.E.M., N = 5

TABLE 17. MEAN VALUES[a] OF SERUM BIOCHEMISTRY PARAMETERS FOR MALE NZW RABBITS FOLLOWING 21-DAY REPEATED DERMAL TREATMENT WITH CTP

Parameter	Control	0.25 mg/L	0.50 mg/L	1.00 mg/L
BUN (mg/dL)				
Pre-exposure	18.08 ± 1.26[b]	14.50 ± 0.87	16.25 ± 1.98	15.82 ± 0.51
Postexposure	16.40 ± 0.70	13.60 ± 0.30	16.04 ± 0.89	14.88 ± 1.05
Creatinine (mg/dL)				
Pre-exposure	1.23 ± 0.08[b]	0.94 ± 0.04	1.00 ± 0.07	1.16 ± 0.06
Postexposure	0.66 ± 0.05	0.80 ± 0.05	1.00 ± 0.04	0.98 ± 0.05
Phosphorus (mg/dL)				
Pre-exposure	6.99 ± 0.24[b]	6.76 ± 0.10	6.63 ± 0.03	7.88 ± 1.05
Postexposure	6.29 ± 0.11	6.01 ± 0.27	5.49 ± 0.51	5.88 ± 0.23
Calcium (mg/dL)				
Pre-exposure	14.83 ± 0.33[b]	14.26 ± 0.39	14.90 ± 0.32	15.38 ± 0.32
Postexposure	14.24 ± 0.26	14.70 ± 0.34	14.88 ± 0.16	15.04 ± 0.21
Total Protein (g/dL)				
Pre-exposure	6.00 ± 0.11[b]	5.92 ± 0.08	6.00 ± 0.09	6.07 ± 0.15
Postexposure	5.25 ± 0.45	5.99 ± 0.16	5.70 ± 0.42	6.08 ± 0.20
Alk. Phos. (IU/L)				
Pre-exposure	275.00 ± 28.59[b]	242.40 ± 12.89	209.75 ± 31.11	220.80 ± 18.19
Postexposure	175.20 ± 18.91	247.00 ± 22.35	269.80 ± 55.16	213.60 ± 25.51
SGOT (IU/L)				
Pre-exposure	41.75 ± 20.22[b]	29.80 ± 2.08	36.75 ± 5.94	33.00 ± 5.38
Postexposure	50.20 ± 1.66	43.40 ± 6.80	29.60 ± 2.11	34.40 ± 4.34
SGPT (IU/L)				
Pre-exposure	14.00 ± 4.00[c]	27.75 ± 6.42	125.00 ± 95.00[c]	19.20 ± 5.03
Postexposure	28.20 ± 3.41	19.80 ± 4.47	15.75 ± 3.79	22.50 ± 5.07
LDH				
Pre-exposure	----[d]	226.00 ± 21.00	74.00 ± < 0.01	----[d]
Postexposure	98.50 ± 25.96	168.60 ± 46.98	191.00 ± 29.41	89.20 ± 18.66

[a] Mean ± S.E.M., N = 5 except as noted
[b] N = 4
[c] N = 2
[d] Insufficient sample

TABLE 18. MEAN VALUES[a] OF SERUM BIOCHEMISTRY PARAMETERS FOR FEMALE NZW RABBITS FOLLOWING 21-DAY REPEATED DERMAL TREATMENT WITH CTP

Parameter	Control	0.25 mg/L	0.50 mg/L	1.00 mg/L
BUN (mg/dL)				
Pre-exposure	20 94 ± 1 06	18 96 ± 1 72	18 32 ± 0 84	18 04 ± 1 18
Postexposure	18 34 ± 1 37	18 58 ± 1 77	19 50 ± 0 72	18 48 ± 0 78
Creatinine (mg/dL)				
Pre-exposure	0 90 ± 0 09	1 18 ± 0 05	1 10 ± < 0 01	1 15 ± 0 03[b]
Postexposure	1 12 ± 0 08	1 12 ± 0 04	0 98 ± 0 02	1 06 ± 0 05
Phosphorus (mg/dL)				
Pre-exposure	6 28 ± 0 41	6 82 ± 0 17	6 43 ± 0 42	6 48 ± 0 23
Postexposure	5 56 ± 0 51	5 86 ± 0 20	5 98 ± 0 25	5 40 ± 0 21
Calcium (mg/dL)				
Pre-exposure	15 14 ± 0 12	15 70 ± 0 23	15 50 ± 0 21	15 48 ± 0 20
Postexposure	14 00 ± 0 48	13 82 ± 0 17	13 75 ± 0 75	----[d]
Total Protein (g/dL)				
Pre-exposure	6 12 ± 0 17	6 06 ± 0 12	6 08 ± 0 09	6 27 ± 0 14
Postexposure	6 38 ± 0 25[b]	5 80 ± 0 10[c]	5 98 ± 0 08	----[d]
Alk Phos (IU/L)				
Pre-exposure	219 00 ± 13 83	228 00 ± 7 26	284 60 ± 27 51	262 20 ± 42 96
Postexposure	180 60 ± 9 11	198 60 ± 8 68	257 00 ± 44 91	229 80 ± 29 27
SGOT (IU/L)				
Pre-exposure	48 80 ± 6 53	36 00 ± 2 43	40 25 ± 4 57[b]	39 20 ± 8 70
Postexposure	32 40 ± 2 16	33 40 ± 6 50	32 40 ± 4 25	37 00 ± 12 08
SGPT (IU/L)				
Pre-exposure	54 00 ± 8 26[b]	66 00 ± 12 15[b]	53 75 ± 13 22[b]	46 40 ± 12 79
Postexposure	42 60 ± 11 04	40 60 ± 10 20	28 40 ± 4 59	26 20 ± 5 57
LDH				
Pre-exposure	103 67 ± 32 63[c]	179 00 ± 30 28[b]	216 00 ± 10 79[b]	231 40 ± 20 47
Postexposure	129 00 ± 17 04	117 40 ± 20 23	149 40 ± 18 45	217 00 ± 20 72

[a] Mean ± S E M N = 5 except as noted
[b] N = 4
[c] N = 3
[d] Insufficient sample

Statistical analysis of the organ weights, measured at necropsy (Tables 19, 20), identified the kidneys, liver, and thymus of selected test rabbit groups as being different from those organ weights in the respective control group The absolute kidney weights and the kidney to body weight ratios were increased in both sexes dosed with 0 25 g/kg and the organ to body weight ratio was increased in the male rabbits dosed with 0 5 g CTP/kg Although these values for the kidneys from the highest dose group were greater than those of controls, the differences were not significant ($p > 0\ 05$) Similar results were observed for the liver Significantly increased absolute liver weights were observed in the 0 25 and 0 50 g/kg groups of both sexes, while the difference between the control and high-dose group was not significant The increased liver weights observed resulted in an increased ($p < 0\ 05$) liver to body weight ratio in the male rabbits dosed with 0 50 g CTP/kg The high-dose male rabbit group exhibited a mean thymus weight and mean thymus to body weight ratio significantly lower than the male control group ($p < 0\ 01$)

TABLE 19. MEAN ORGAN WEIGHTS[a] (g) AND ORGAN TO BODY WEIGHT RATIOS (%) OF MALE NZW RABBITS FOLLOWING 21-DAY REPEATED DERMAL TREATMENT WITH CTP

Parameter	Control[b]	0.25 mg/L[c]	0.50 mg/L[b]	1.00 mg/L[c]
Adrenals	0.22 ± 0.02	0.21 ± 0.01	0.22 ± 0.01	0.21 ± 0.02
Ratio[d]	0.01 ± < 0.01	0.01 ± < 0.01	0.01 ± < 0.01	0.01 ± < 0.01
Brain	8.93 ± 0.11	9.16 ± 0.21	9.16 ± 0.23	9.05 ± 0.19
Ratio	0.30 ± 0.01	0.29 ± 0.01	0.31 ± 0.01	0.30 ± 0.01
Heart	8.84 ± 0.61	8.99 ± 0.60	9.56 ± 0.65	9.26 ± 0.75
Ratio	0.30 ± 0.02	0.29 ± 0.02	0.32 ± 0.02	0.31 ± 0.02
Kidney	17.50 ± 0.88	20.26 ± 1.14[e]	19.30 ± 0.74	19.05 ± 0.82
Ratio	0.59 ± 0.02	0.64 ± 0.03[f]	0.65 ± 0.02[f]	0.63 ± 0.01
Liver	96.83 ± 3.29	123.40 ± 8.93[f]	123.52 ± 7.87[f]	117.81 ± 6.87
Ratio	3.27 ± 0.09	3.92 ± 0.23	4.14 ± 0.20[f]	3.89 ± 0.18
Lung	25.08 ± 2.61	25.90 ± 2.62	27.93 ± 3.16	27.97 ± 2.83
Ratio	0.85 ± 0.10	0.84 ± 0.10	0.95 ± 0.12	0.94 ± 0.11
Testes	3.38 ± 0.17	3.39 ± 0.27	3.33 ± 0.27	3.64 ± 0.24
Ratio	0.11 ± < 0.01	0.11 ± 0.01	0.11 ± 0.01	0.12 ± 0.01
Spleen	0.93 ± 0.05	0.99 ± 0.10	0.90 ± 0.08	0.96 ± 0.07
Ratio	0.03 ± < 0.01	0.03 ± < 0.01	0.03 ± < 0.01	0.03 ± < 0.01
Thymus	4.13 ± 0.33	4.32 ± 0.39	4.17 ± 0.33	2.55 ± 0.75[e]
Ratio	0.14 ± 0.01	0.14 ± 0.01	0.14 ± 0.01	0.08 ± 0.02[e]
Whole body (kg)	**2.96 ± 0.07**	**3.14 ± 0.09**	**2.97 ± 0.08**	**3.02 ± 0.08**

[a] Mean ± S.E.M.
[b] N = 10
N = 9
[d] Organ weight/body weight × 100
[e] Significantly different from controls at $p < 0.01$ using a two factorial analysis of variance with multivariate comparisons
[f] Significantly different from controls at $p < 0.05$ using a two factorial analysis of variance with multivariate comparisons

TABLE 20. MEAN ORGAN WEIGHTS[a] (g) AND ORGAN TO BODY WEIGHT RATIOS (%) OF FEMALE NZW RABBITS FOLLOWING 21-DAY REPEATED DERMAL TREATMENT WITH CTP

Parameter	Control	0.25 mg/L	0.50 mg/L	1.00 mg/L
Adrenals	0.24 ± 0.01	0.26 ± 0.02	0.21 ± 0.01	0.26 ± 0.02
Ratio[b]	0.01 ± < 0.01	0.01 ± 0.01	0.01 ± < 0.01	0.01 ± < 0.01
Brain	8.79 ± 0.11	8.98 ± 0.18	8.84 ± 0.13	8.69 ± 0.19
Ratio	0.30 ± 0.01	0.28 ± 0.01	0.28 ± 0.01	0.29 ± 0.01
Heart	8.40 ± 0.54	10.04 ± 0.66	8.84 ± 0.59	8.92 ± 0.57
Ratio	0.29 ± 0.02	0.31 ± 0.02	0.28 ± 0.02	0.29 ± 0.02
Kidney	16.55 ± 0.82	19.92 ± 0.52[c]	18.50 ± 0.59	17.88 ± 0.83
Ratio	0.56 ± 0.02	0.61 ± 0.02[c]	0.59 ± 0.02	0.59 ± 0.02
Liver	107.69 ± 4.14	123.57 ± 6.31[c]	118.74 ± 7.66[c]	109.89 ± 7.50
Ratio	3.64 ± 0.09	3.79 ± 0.13	3.79 ± 0.21	3.61 ± 0.19
Lung	26.44 ± 2.62	24.46 ± 1.73	28.71 ± 2.90	32.60 ± 1.43
Ratio	0.90 ± 0.09	0.76 ± 0.06	0.92 ± 0.09	1.08 ± 0.05
Ovaries	0.21 ± 0.02	0.22 ± 0.01	0.20 ± 0.01	0.20 ± 0.02
Ratio	0.01 ± < 0.01	0.01 ± < 0.01	0.01 ± < 0.01	0.01 ± < 0.01
Spleen	0.99 ± 0.07	1.10 ± 0.08	1.02 ± 0.07	1.12 ± 0.05
Ratio	0.03 ± < 0.01	0.03 ± < 0.01	0.03 ± < 0.01	0.04 ± < 0.01
Thymus	3.44 ± 0.18	4.30 ± 0.30	3.96 ± 0.36	3.96 ± 0.24
Ratio	0.12 ± 0.01	0.13 ± 0.01	0.13 ± 0.01	0.13 ± 0.01
Whole body (kg)	**2.95 ± 0.06**	**3.25 ± 0.07**	**3.13 ± 0.06**	**3.03 ± 0.09**

[a] Mean ± S.E.M. N = 10
[b] Organ weight/body weight × 100
[c] Significantly different from controls at $p < 0.05$ using a two factorial analysis of variance with multivariate comparisons

There were no CTP treatment related lesions identified in either sex of dosed rabbits However, several pathologic findings were noteworthy and are presented as background information on these animals Gross examinations and subsequent histopathologic examinations disclosed one low-dose CTP-treated male rabbit to have a vertebral fracture, one median dose CTP-treated male rabbit to have pampiniform plexus congestion, and one median dose CTP-treated female rabbit to have liver necrosis Subacute typhilitis, involving from 56 to 100% of each dose/sex group, high incidences of cecal pinworms (*Passalurus ambiguus*), mesenteric and ileal lymphoid hyperplasia, and subacute ileitis were detected Scattered cases (four total) of pulmonary abscessation occurred The abscesses were consistent with those associated with Pasteurellosis Pulmonary edema was diagnosed in two or fewer male rabbits at each of the three CTP doses; and in three female controls, two females from the median CTP dose group, and one female in the high CTP dose group (Table 21) Fifty percent of the female rabbits in the control, median CTP dose, and high CTP dose groups had pulmonary congestion. The incidence of pulmonary congestion was 20% or less in all other rabbit dose/sex groups Renal tubular mineralization occurred in one male rabbit from each dose group, including controls, and in one, five, four, and three female rabbits from the controls, low, median and high CTP dose groups, respectively Statistical analyses indicated that female rabbits had higher incidences of ileal and cecal subacute inflammation ($p < .01$), dilated renal tubules ($p < .05$), renal tubular mineralization ($p < .05$), mandibular lymph node lymphoid hyperplasia ($p < .01$), and pulmonary congestion ($p < .01$) when compared to the male rabbit groups; however, increases in incidences of histologic lesions were not dose-related

TABLE 21. INCIDENCE (%) SUMMARY OF SELECTED MICROSCOPIC LESIONS OF RABBITS FOLLOWING 21-DAY DERMAL TREATMENT WITH CTP

	Males				Females			
Organ – Lesion	Control	0.25 mg/kg	0.50 mg/kg	1.00 mg/kg	Control	0.25 mg/kg	0.50 mg/kg	1.00 mg/kg
Lung								
Pulmonary Edema	0	20	10	10	30	0	20	10
Pulmonary Congestion	20	10	0	0	50	10	50	50
Kidney								
Tubular Mineralization	10	10	10	10	10	50	40	30
Dilated Tubules	0	0	0	0	30	30	0	0
Ileum								
Subacute Inflammation	0	20	0	13	50	50	44	11
Cecum								
Subacute Inflammation	100	70	56	56	90	90	100	100
Mandibular lymph node								
Lymphoid hyperplasia	50	33	50	0	40	40	22	20

SECTION 4

DISCUSSION

Repeated inhalation of this hydraulic fluid resulted in a transitory depression in body weight gains in both male and female rats during the first week of exposure; however, body weight gains of the treated rat groups were comparable to their respective control groups during the final two weeks of the study. No treatment-related effects were noted in the body weights of rabbits during the 21-day dermal study. Gross examinations of both rats and rabbits at the conclusion of the studies failed to reveal any treatment-related lesions.

Spleen, liver, and thymus weight differences noted in the test animals were, for the most part, not treatment-related nor were pathological changes borne out in the microscopic examination of those tissues. Relative testes weights of the test rats were significantly greater than those of the controls, but the differences directly paralleled that noted in whole body weights. However, absolute testes weights were comparable among groups.

Based on the analysis of incidence and severity data, the only inhalation-related effect was pulmonary alveolar histiocytosis, most severe in high-dose male and female rats, and renal tubule hyaline droplet accumulation. Although there was a high incidence of hyaline droplet accumulation in the renal tubule epithelium of male and female rats, these lesions were minimal to mild, and often represented background lesions, especially in male rats. The lack of hyaline droplet accumulation in the controls, however, suggested that CTP exposure may trigger hyaline droplet accumulation. The increased number of lung macrophages was not associated with detectable injury to lung structure and was most likely due to a heightened pulmonary clearance response. The apparent mildness of the lung and renal lesions suggested that CTP had little inhalation toxicity in rats at the concentrations tested.

The fact that CTP could not be detected in blood or urine following aerosol inhalation (Kinkead and Bashe, 1987) and the more abundant pulmonary macrophages demonstrated in the present study suggest that CTP may be cleared from the lung by alveolar macrophage phagocytic activity and mucociliary clearance. Although oily materials may be absorbed by the lung, the most frequently observed morphologic response is an increase in alveolar macrophages that become laden with the phagocytized material. Lipid pneumonia, a granulomatous lung disease associated with the aspiration of oils, is characterized by phagocytosis of emulsified oil (Robbins and Angell, 1976). The more unsaturated oils tend to cause the greater irritant effect in the lung. Since macrophages cleared from the lung are swallowed when they arrive at the nasopharynx, studies that compare blood and urine levels of CTP with CTP levels in lung macrophages and feces at varied postexposure intervals may be helpful for assessing the fate of inhaled aerosolized CTP.

None of the pathologic effects observed in rabbits were attributed to CTP exposure Congestion and edema in the respiratory tract were considered agonal or postmortem changes Other lesions represent bacterial infection (pulmonary abscesses), parasitic infection (typhilitis, ileitis, colitis, lymphadenitis, mesenteric and ileal hyperplasia), or mild background lesions which did not confound the interpretation of study results

SECTION 5

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APPENDIX 1

INDIVIDUAL BODY WEIGHTS (g) OF F-344 RATS DURING 21-DAY REPEATED INHALATION EXPOSURE TO CTP

Dose Group	Sex	Animal No.	Body Weights (g)			
			Day 0	Day 7	Day 14	Day 21*
Control	Male	0130003	215	233	246	243
		0130005	204	219	240	238
		0130009	206	222	231	227
		0130023	169	222	230	222
		0130029	174	225	243	237
		0130030	170	222	239	236
		0130031	213	231	242	236
		0130038	215	234	248	239
		0130039	216	232	247	239
		0130043	206	221	236	224
		Mean ± S.E.M	**199 ± 6**	**226 ± 2**	**240 ± 2**	**234 ± 2**
0.25 mg/L	Male	0130002	207	211	228	222
		0130007	193	205	221	218
		0130008	211	221	238	228
		0130011	169	209	224	215
		0130019	182	225	244	239
		0130022	169	211	227	219
		0130032	193	205	211	206
		0130036	207	213	229	224
		0130044	204	216	232	225
		0130047	201	209	227	225
		Mean ± S.E.M.	**194 ± 5**	**213 ± 2**	**228 ± 3**	**222 ± 3**
0.50 mg/L	Male	0130001	209	214	226	222
		0130004	199	205	212	206
		0130010	214	221	235	232
		0130012	214	222	235	225
		0130013	208	215	234	226
		0130015	211	215	228	225
		0130024	219	219	235	228
		0130027	203	204	219	214
		0130035	225	228	242	238
		0130040	198	203	219	211
		Mean ± S.E.M.	**210 ± 3**	**215 ± 3**	**229 ± 3**	**223 ± 3**
1.00 mg/L	Male	0130006	209	219	238	229
		0130016	204	214	223	215
		0130017	195	206	223	215
		0130018	202	209	224	220
		0130021	201	206	228	224
		0130025	182	199	212	213
		0130034	191	202	218	211
		0130037	208	209	222	220
		0130041	182	191	207	203
		0130042	215	217	233	228
		Mean ± S.E.M.	**199 ± 4**	**207 ± 3**	**223 ± 3**	**218 ± 3**

* Fasted weights

(continued)

APPENDIX 1. (continued)

Dose Group	Sex	Animal No.	Body Weights (g) Day 0	Day 7	Day 14	Day 21*
Control	Female	0130050	151	154	165	156
		0130059	159	159	165	155
		0130063	146	151	160	151
		0130067	145	148	153	140
		0130078	141	144	151	140
		0130080	141	146	155	143
		0130084	145	150	155	145
		0130088	142	144	147	136
		0130089	150	155	159	152
		0130092	152	154	156	150
		Mean ± S.E.M.	**147 ± 2**	**151 ± 2**	**157 ± 2**	**147 ± 2**
0 25 mg/L	Female	0130051	149	145	152	144
		0130052	158	158	160	152
		0130056	146	149	152	147
		0130057	145	141	145	138
		0130061	151	152	156	147
		0130064	149	144	156	148
		0130074	156	150	156	151
		0130075	147	143	148	139
		0130083	154	152	156	149
		0130091	153	148	153	147
		Mean ± S.E.M.	**151 ± 1**	**148 ± 2**	**153 ± 1**	**146 ± 2**
0 50 mg/L	Female	0130048	145	143	145	139
		0130054	145	149	152	144
		0130060	142	139	140	137
		0130065	147	144	150	146
		0130070	141	136	142	140
		0130071	137	133	132	129
		0130072	159	158	163	157
		0130076	154	149	155	153
		0130079	152	144	148	145
		0130085	150	147	152	143
		Mean ± S.E.M.	**147 ± 2**	**144 ± 2**	**148 ± 3**	**143 ± 3**
1 00 mg/L	Female	0130049	167	165	168	164
		0130062	146	143	150	144
		0130066	141	141	147	144
		0130069	142	139	147	145
		0130073	144	143	149	141
		0130077	139	135	142	134
		0130081	145	144	147	141
		0130082	152	149	155	153
		0130087	146	139	145	140
		0130090	153	153	159	149
		Mean ± S.E.M.	**148 ± 3**	**145 ± 3**	**151 ± 3**	**146 ± 3**

* Fasted weights

APPENDIX 2

INDIVIDUAL BODY WEIGHTS (kg) OF NZW RABBITS DURING 21-DAY REPEATED DERMAL TREATMENT WITH CTP

Dose Group	Sex	Animal No.	Body Weights (kg)			
			Day 0	Day 7	Day 14	Day 21*
Control	Male	T40	2.6	2.6	2.6	2.7
		T50	2.7	2.9	2.9	2.9
		T54	3.0	3.0	3.2	3.2
		T60	2.8	2.9	3.0	3.0
		T70	2.6	2.6	2.6	2.7
		T80	2.6	2.6	2.6	2.8
		T82	2.6	2.6	2.7	2.8
		T84	3.0	3.1	3.3	3.4
		T92	2.7	2.9	3.0	3.1
		U08	2.7	2.8	2.8	3.0
		Mean ± S.E.M.	**2.7 ± 0.1**	**2.8 ± 0.1**	**2.9 ± 0.1**	**3.0 ± 0.1**
0.25 g/kg	Male	T34	2.8	3.0	3.2	3.3
		T36	2.9	3.1	3.2	3.3
		T52	2.6	2.6	2.6	2.6
		T72	2.9	2.9	3.1	3.0
		T76	2.7	2.8	2.8	2.9
		T88	2.5	2.5	2.5	---
		T96	2.8	2.9	3.0	3.2
		U04	2.9	3.0	3.1	3.3
		U14	3.1	3.1	3.2	3.5
		U18	2.9	2.9	3.1	3.2
		Mean ± S.E.M.	**2.8 ± 0.1**	**2.9 ± 0.1**	**3.0 ± 0.1**	**3.1 ± 0.1**
0.50 g/kg	Male	T38	2.9	3.1	3.2	3.4
		T42	2.5	2.6	2.7	2.7
		T44	2.5	2.6	2.6	2.6
		T48	2.6	2.8	3.0	3.0
		T66	2.9	3.0	3.1	3.2
		T68	2.4	2.7	2.9	2.9
		T74	2.6	2.7	2.8	2.9
		T78	2.6	2.6	2.7	2.8
		U02	2.6	2.7	2.8	3.0
		U10	2.9	3.1	3.2	3.2
		Mean ± S.E.M.	**2.7 ± 0.1**	**2.8 ± 0.1**	**3.0 ± 0.1**	**3.0 ± 0.1**
1.00 g/kg	Male	T32	2.6	2.7	2.8	2.8
		T62	2.5	2.6	2.7	2.8
		T86	2.9	3.1	3.2	3.2
		T90	2.8	2.9	2.9	3.1
		T94	2.8	3.0	3.1	3.0
		T98	2.5	2.5	2.5	2.6
		U06	3.0	3.0	3.1	3.1
		U12	2.9	3.0	3.2	3.3
		U20	2.7	---	---	---
		U22	2.9	3.0	3.1	3.3
		Mean ± S.E.M.	**2.8 ± 0.1**	**2.9 ± 0.1**	**3.0 ± 0.1**	**3.0 ± 0.0**

* Fasted weights

(continued)

APPENDIX 2. (continued)

Dose Group	Sex	Animal No.	Body Weights (kg) Day 0	Day 7	Day 14	Day 21*
Control	Female	X31	2.8	2.7	2.8	3.0
		X37	2.6	2.7	2.8	2.9
		X55	2.7	2.6	2.7	2.7
		X61	2.7	2.8	2.9	3.0
		X83	2.8	3.0	3.1	3.3
		X87	2.5	2.6	2.7	2.8
		X99	2.7	3.0	3.1	3.2
		Y07	2.6	2.8	2.7	2.8
		Y15	2.7	2.8	2.9	2.9
		Y19	2.6	2.6	2.9	2.9
		Mean ± S.E.M.	2.7 ± 0.0	2.8 ± 0.1	2.9 ± 0.1	3.0 ± 0.1
0.25 g/kg	Female	X33	3.0	3.0	3.1	3.2
		X51	2.7	2.6	2.8	2.9
		X57	3.0	3.1	3.4	3.6
		X59	2.9	3.0	3.1	3.2
		X71	2.8	2.9	3.0	3.2
		X75	3.1	3.3	3.5	3.6
		X77	2.8	2.8	3.0	3.2
		X81	2.8	2.7	2.9	3.0
		X89	2.8	3.0	3.2	3.4
		X91	2.8	2.9	3.1	3.2
		Mean ± S.E.M.	2.9 ± 0.0	2.9 ± 0.1	3.1 ± 0.1	3.3 ± 0.1
0.50 g/kg	Female	X35	2.7	2.8	2.8	3.1
		X39	2.7	2.7	2.7	3.0
		X47	2.7	2.8	2.8	3.0
		X53	2.6	2.9	3.1	3.3
		X67	2.9	2.9	3.1	3.3
		X73	2.8	2.8	2.8	2.9
		X97	2.8	2.6	2.8	2.8
		Y05	2.9	3.0	3.1	3.3
		Y13	2.9	2.9	3.2	3.2
		Y17	3.1	3.2	3.3	3.4
		Mean ± S.E.M.	3.1 ± 0.1	2.9 ± 0.1	3.0 ± 0.1	3.1 ± 0.1
1.00 g/kg	Female	X41	2.9	3.0	3.1	3.3
		X45	2.9	2.9	3.0	3.4
		X63	2.9	2.9	2.9	3.1
		X65	2.6	2.7	2.7	3.0
		X79	2.7	2.8	2.9	3.1
		X85	2.5	2.5	2.6	2.6
		X93	2.6	2.6	2.7	2.8
		X95	3.0	3.0	3.0	3.3
		Y03	2.6	2.6	2.5	2.6
		Y09	2.9	2.9	3.0	3.1
		Mean ± S.E.M.	2.8 ± 0.1	2.8 ± 0.1	2.8 ± 0.1	3.0 ± 0.1

* Fasted weights

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QUALITY ASSURANCE

The study, "Determination of the Toxicity of Cyclotriphosphazene Hydraulic Fluid by 21 Day Repeated Inhalation and Dermal Exposure," was conducted by the NSI Technology Services Corporation, Toxic Hazards Research Unit, under the guidance of the Environmental Protection Agency's Good Laboratory Practices Guidelines, 40CFR PART 792. The various phases of this study were inspected by members of the Quality Assurance Unit. Results of these inspections were reported directly to the Study Director at the close of each inspection.

DATE OF INSPECTION:	ITEM INSPECTED:
March 4, 1987	Study protocol
July 9, 1987	21-Day inhalation exposure preparation, initiation
July 21, 1987	Interim data audit, 21-day inhalation exposure
July 29, 1987	Scheduled sacrifice, 21-day inhalation exposure
August 3, 1987	Dermal exposure blood samples
August 4, 1987	Dermal exposure animal dosing
August 11, 1987	Chemistry data
August 25, 1987	Scheduled sacrifice, 21-day dermal exposure
December 5, 1988, January 3, 1989	Final report and data audit

The Quality Assurance Unit has determined by review process that this report accurately describes those methods and standard operating procedures required by the protocol and that the reported results accurately reflect the raw data obtained during the course of the study. No discrepancies were found that would alter the interpretation presented in this Final Report.

M G Schneider
QA Coordinator
Toxic Hazards Research Unit

Date March 27, 1989