SUBACUTE AND CHRONIC TOXICITY STUDIES OF TRIETHYLENETETRAMINE DIHYDROCHLORIDE (TJA-250) BY ORAL ADMINISTRATION TO F-344 RATS

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ABSTRACT—Triethylenetetramine dihydrochloride (trientine-2HCl, TJA-250), a copper chelating agent used to treat Wilson's disease, was administered orally to male and female F-344 rats for 4 or 8 weeks at dosages of 0, 100, 350 or 1200 mg/kg/day or for 26 weeks at dosages of 50, 175 or 600 mg/kg/day.

4 or 8-week study. Two males receiving 1200 mg/kg/day died during week 8 of treatment. In males receiving 1200 mg/kg/day during weeks 5 to 8 of treatment, body weight gain and food consumption were decreased and hunched posture and thin build were observed. During week 4 or 8 of treatment urinalysis revealed, for males receiving 100 mg/kg/day or animals receiving 350 mg/kg/day or more, increased electrolyte outputs possibly due to the hydrochloride nature of trientine-2HCl, with low plasma alkaline phosphatase activities evident in animals receiving 350 or 1200 mg/kg/day. After 4 and 8 weeks, and during 8 weeks of treatment, high lung weights and bronchiolar epithelium hypertrophy and broncho-alveolar pneumonia were recorded for animals receiving 1200 mg/kg/day, and submucosal acute inflammation within the glandular region of the stomach was recorded for males receiving 350 or 1200 mg/kg/day and in all treated female groups.

26-week study. One male receiving 175 mg/kg/day and three males receiving 600 mg/kg/day died, showing lung changes. The body weight gain of animals receiving 600 mg/kg/day was slightly decreased. Blood chemistry and urinalysis examinations showed changes similar to those indicated in the 4- or 8-week study. The low plasma copper concentrations seen in males receiving 600 mg/kg/day, the slightly low liver copper concentrations found in animals receiving 600 or 175 mg/kg/day and the high urinary copper concentrations found in all treated groups, are attributed to the pharmacological action of trientine-2HCl. Histopathology revealed a dosage-related incidence and severity of focal chronic interstitial pneumonitis accompanied by fibrosis of the alveolar walls in females receiving 175 mg/kg/day or more and all treated male groups, but no significant pathological changes in the stomach. Apart from the histological changes found in the lung, all the above changes were reversible.

In conclusion, the NOAEL of trientine-2HCl in this 26-week study was considered to be 50 mg/kg/day for females and less than 50 mg/kg/day for males.

KEY WORDS: Triethylenetetramine dihydrochloride, Copper chelating agent, Wilson's disease, Repeated dose toxicity, Interstitial pneumonitis, Rat

INTRODUCTION

Triethylenetetramine dihydrochloride, TJA-250 (trientine-2HCl) is used as a chelating agent for treatment of Wilson's disease, a rare, inherited metabolic disease characterized by an excessive accumulation of copper in certain tissues. This causes findings such as hepatic cirrhosis, extrapyramidal sign and Kayser-Fleischer ring. Penicillamine is the first medical chelating agent which was successfully introduced for treatment of the disease (Walshe, 1956). However, about 10 % of patients using penicillamine eventually develop some form of serious immunological intolerance to the drug (Walshe, 1982). Walshe found another chelating agent, trientine-2HCl, which was effective in managing the disease in patients who have developed this intolerance to penicillamine (Walshe, 1969). The agent was designated as an orphan drug and approved for use in these patients by the US FDA and MHW in Japan.

There are two reports in which subchronic toxicity of trientine was described. Yamaguchi (1992) administered trientine-4HCl orally by gavage to Wistar/ST rats at dosages up to 571 (males) or 633 (females) mg/kg/day for 90 days and reported that no obvious dose-dependent changes were observed at hematological, blood chemical and pathological examinations in both sexes. Greenman *et al.* (1996) evaluated trientine-2HCl for subchronic toxicity when given in drinking water at concentrations up to 3000 ppm to F-344 rats and B6C3F₁ mice fed either a cereal-based or purified diet over a period of 90 days. Trientine-2HCl toxicity occurred only in mice in the highest dose group fed a purified diet. Increased frequencies of inflammation of the lung interstitium and liver periportal fatty infiltration were seen in both sexes, and hematopoietic cell proliferation was seen in the spleen of males. However, no chronic toxicity was reported for trientine. The pur-

pose of this study was to characterize the possible toxicity of trientine-2HCl when administered to F-344 rats for up to 26 weeks and to evaluate the reversibility of any effect.

MATERIALS AND METHODS

Test material

Triethylenetetramine dihydrochloride, TJA-250, N,N'-bis(2-aminoethyl)-1,2-ethanediamine dihydrochloride (trientine-2HCl) is a white to pale yellow crystalline powder easily soluble in water. The chemical structure of trientine-2HCl is shown in Fig. 1. Chemical analyses showed that trientine-2HCl was stable during each dosing period. Trientine-2HCl was dissolved in reverse osmosis water and shown to be stable in reverse osmosis water for at least 96 hr at both ambient temperature and at 4°C. All formulations were prepared freshly each day.

Animals and housing

Male and female Fischer (F-344) rats were obtained from Charles River (UK) Limited, Kent, England and allowed to acclimatize appropriately for 5 days in a 4- or 8-week study or for 14 days in a 26-week study. The animals were housed four or five of one sex per cage in stainless steel cages (54×38×20 cm) in animal rooms which were maintained at target values of 21°C for temperature, 55% for relative humidity (actual ranges were 18.5-25.5°C and 42-66 %) and at least 14 air changes per hour and provided

Fig. 1. Chemical structure of N,N'-bis(2-aminoethyl)-1,2-ethanediamine dihydrochloride.

Table 1. Experimental design of 4- or 8-week and 26-week toxicity studies of trientine-2HCl.

4- or 8-week study			26-week study		
Dosage	No. of animals / sex		Dosage	No. of animals / sex	
(mg/kg/day)	4 weeks	8 weeks	(mg/kg/day)	Main study	Reversibility study
0	5	5	0	12	8
100	5	5	50	12	0
350	5	5	175	12	0
1200	5	5	600	12	8

with lighting on a 12-hr light/dark cycle (on at 6 a.m. off at 6 p.m.). The animals were given free access to an expanded rodent diet, RM1(E) SQC (Special Diets Services Ltd., Essex, England) and tap water.

Study design and administration

The experimental design of the studies is presented in Table 1. The duration of the 4- or 8-week study was intended initially for 4 weeks. No obvious toxic effects were observed on body weight gain during 4 weeks of treatment and on clinical examinations performed during week 4 of treatment. In order to further assess possible treatment-related effects, five animals from each group/sex continued treatment for a further 4 weeks. Dosages of trientine-2HCl for the 4- or 8-week study were based on the results of a preliminary 2-week study which showed decreased body weight gain at more than 900 mg/kg/day and that 1350 mg/kg/day was a dose likely to produce adverse effects. In the 26-week study, rats for reversibility study were treated similarly to those for the main study followed by a period of 13 weeks without treatment. Dosages of the 26-week study were determined from the 4- or 8-week study, i.e. 1200 mg/kg/day caused deaths in F-344 rats and exceeded the maximum tolerated dosage (MTD). Animals received trientine-2HCl or vehicle control formulations by gavage at a volume-dosage of 10 ml/kg body weight/day once each day, 7 days per week. Dosing was started at 33 to 42 days of age.

Signs, body weight and food and water consumption

Animals were inspected at least twice daily for evidence of treatment-related signs or ill-health during the first week of treatment, twice weekly during weeks 2 to 4 of treatment and once weekly thereafter. During weeks 14 to 26 and the reversibility period in the 26-week study, animals were inspected once every two weeks. In addition, a more detailed weekly examination, which included palpation, was performed on each animal. Body weight was measured on the day that treatment commenced and at twice weekly intervals throughout the treatment period in the 4- or 8-week study or at weekly intervals throughout the treatment and reversibility periods in the 26-week study. The weight of food supplied to each cage, that remaining, and an estimate of the amount spilled were recorded for each week throughout the treatment period in both studies and the reversibility period in the 26-week study. Water consumption was recorded (over a 3-day period on each occasion), during the first 4 weeks of

treatment in the 4- or 8-week study, during weeks 1, 6, 13 and 25 of treatment and week 13 of the reversibility period in the 26-week study. In addition, during week 8 of treatment in the 4- or 8-week study and during weeks 12 (males), 13 (females) and 25 of treatment and week 13 of the reversibility period in the 26-week study, water consumption measurements were performed for all surviving animals over an approximate 24-hour period, as two separate intervals (after dosing until 5 p.m. [Interval 1] and 5 p.m. until 9 a.m. the following day [Interval 2]). The measurements were performed in conjunction with urine collection.

Ophthalmology and vaginal smears

Before commencement and after 24 days (4- or 8-week study) or after 25 weeks (26-week study) of treatment, both eyes were examined by means of an indirect ophthalmoscope (Keeler's Fison Binocular Indirect Ophthalmoscope, Keeler Ltd., UK).

In the 4- or 8-week study, during weeks 3 and 4 of treatment over 12 consecutive days, the duration and regularity of the estrus cycle of all females was assessed by vaginal smear.

Hematology

Blood samples were obtained from all surviving animals, after overnight starvation, during weeks 4 and 8 of treatment in the 4- or 8-week study and during week 24 (males) or week 25 (females) of treatment and during week 12 of the reversibility period in the 26-week study. Blood samples were withdrawn from the retro-orbital sinus, with the animals held under halothane/nitrous oxide anesthesia, and collected into EDTA or citrate as an anticoagulant. Examination of all samples included the following characteristics: packed cell volume (PCV), hemoglobin (Hb), erythrocyte count (RBC), total leucocyte count (WBC), differential leucocyte count (neutrophils (N), lymphocytes (L), eosinophils, basophils, monocytes and a small proportion of large unstained cells) and platelet count using a Technicon H-1 hematology analyzer, Bayer Ltd., USA, abnormal morphology and unusual cell types (Romanowsky stain) and reticulocyte count (brilliant cresyl blue stain) with light microscopy, prothrombin time (PT), activated partial thromboplastin time (APTT) using an Organon Teknika Coag-A-Mate. Mean cell hemoglobin concentration, mean cell hemoglobin and mean cell volume were calculated.

Blood chemistry

At the same time, and using the same animals as for peripheral hematology, further blood samples were taken and collected into lithium heparin as an anticoagulant. After separation, the plasma was examined in respect to alkaline phosphatase (ALP), alanine amino-transferase (ALT), aspartate amino-transferase (AST), creatinine phosphokinase (CPK), lactate dehydrogenase (LDH), urea (Urea), creatinine (Crea), glucose (Gluc), total bilirubin (TBil), triglyceride (Trig), total cholesterol (Chol), total protein (TP), sodium (Na), potassium (K), chloride (Cl), calcium (Ca) and inorganic phosphorus (P) using a Technicon Axon System, Toshiba Ltd., Japan, and electrophoretic protein fractions using cellulose acetate strips.,

Urinalysis

In the 4- or 8-week study overnight during week 4 of treatment (approximately 5 p.m. until 9 a.m. the following morning) urine samples were collected from all animals in an individual metabolism cage without food or water. The individual samples were examined in respect to appearance and volume, pH by a pH meter, specific gravity (SG) using an Atago refractometer, protein by precipitation with sulphosalicylic acid, total reduction substances by Clinitest, glucose, ketones, bilirubin, urobilinogen, nitrite and blood by Multistix, the sediment from centrifugation with microscopy, sodium (Na), potassium (K) and chloride (Cl) using the Technicon Axon System, Toshiba Ltd., Japan. During week 8 of treatment in the 4- or 8-week study and during weeks 12 and 25 of treatment and during week 13 of the reversibility period in the 26-week study, urine was collected from all surviving animals over approximately 24 hr, as two separate intervals (after dosing until 5 p.m. [Interval 1] and 5 p.m. until 9 a.m. the following day [Interval 2]). The urine collections were performed in conjunction with water consumption measurements. The Interval 1 sample was analyzed in respect to volume, sodium, potassium and chloride concentrations in the 4- or 8-week study and, additionally, appearance and pH were measured in the 26-week study. The Interval 2 sample was examined in respect to all of those parameters described above.

Metal analysis

In the 26-week study, concentrations of copper, zinc and iron in plasma, urine, liver and kidney were analyzed. During week 26 of treatment and week 13 of the reversibility period, overnight urine samples were collected from all surviving animals in an individual

metabolism cage without food and water (approximately 5 p.m. until 9 a.m. the following morning). Immediately before necropsy, blood samples were obtained from all surviving animals via the retro-orbital sinus and collected into sodium heparin as an anticoagulant. At necropsy, sections of the liver (left and median lobes) and right kidney (cranial pole) were taken. The plasma, urine, liver and kidney samples were stored deep-frozen (-20°C) until analysis. Five animals in each group were examined at the end of each period. Samples of plasma or urine were oxidized and diluted with nitric acid. Concentrations of copper, zinc and iron in their samples were determined by an atomic absorption spectrophotometer (Perkin Elmer Model 280). Samples of liver or kidney tissues were destroyed and oxidized by heating in nitric acid and perchloric acid and diluted with hydrochloric acid. Measurements of the elements of these diluted samples were made using an inductively coupled plasma emission optical spectrometer (Thermo Electron Plasma 300).

Macroscopic pathology

On completion of the treatment/reversibility period, all surviving animals were killed for detailed necropsy by exsanguination under halothane/nitrous oxide anesthesia in the 4- or 8- week study and by carbon dioxide inhalation in the 26-week study. Animals killed or found dead during the treatment period were also subjected to necropsy.

Organ weights

The following organs were dissected and weighed for each animal: adrenals, brain, epididymides, heart, kidneys, liver, lungs with mainstem bronchi, ovaries, pituitary, prostate, submandibular gland, seminal vesicles, spleen, testes, thymus, thyroid with parathyroids (after fixation) and uterus with cervix.

Histopathology

Samples of the following tissues and any grossly abnormal tissues were preserved in 4% neutral buffered formaldehyde, except eyes, optic nerves and Harderian glands, which were placed in Davidson's fluid and subsequently retained in 70% methylated spirit. In the 26-week study, testes and epididymides were initially preserved in Bouin's fluid; adrenals, brain, caecum, colon, duodenum, epididymides, eye and optic nerve, femoral bone and marrow, Harderian glands, heart, ileum, jejunum, kidneys, lachrymal gland, liver, lungs with mainstem bronchi, lymph nodes (mandibular, mesenteric), mammary gland, esophagus, ovaries, pancreas,

pituitary, prostate, rectum, submandibular gland, seminal vesicles, skeletal muscle, skin, spinal cord, spleen, sternum, stomach, testes, thymus, thyroid with parathyroids, tongue, trachea, urinary bladder, uterus with cervix and vagina. Histopathological examination was performed on the above-mentioned tissues from all animals in the control group and the high dosage group. Examination of the lungs and stomach was extended to animals in the remaining dosage groups. Samples of any abnormal tissues were also retained for histopathological examination.

Statistical evaluation

The significance of inter-group differences in hematology, blood chemistry and urinalysis was assessed by Student's *t*-test using a pooled error variance. For organ weights and body weight changes, homogeneity of variance was tested using Bartlett's test. Whenever this was found to be statistically significant, a Behrens-Fisher test was used to perform pairwise comparisons; otherwise a Dunnett's test (Snedecor *et al.*, 1967) was used. Metal analyses were assessed using Dunnett's test. Inter-group differences in macroscopic pathology and histopathology were assessed using Fisher's Exact test (Fisher, 1950).

RESULTS

4- or 8-week study

1. Signs and mortality

Signs considered to be related to treatment with trientine-2HCl were confined to a slightly higher incidence of yellow perigenital staining in females receiving 350 or 1200 mg/kg/day which was usually apparent from the first week of treatment. During the latter half of the treatment period, a hunched posture and thin build was evident in four and three males receiving 1200 mg/kg/day, respectively. During week 8 of treatment, in animals receiving 1200 mg/kg/day, one male was killed *in extremis* and another male was found dead. Previous signs in these animals consisted of thin build, underactive behavior, reduced body temperature, piloerection, pallor, pale eyes, a hunched posture and brown staining around the muzzle. Shallow and irregular respiration was also observed prior to death in the animal that was killed. Increased lung weights and dark lung were recorded at necropsy, and bronchiolar epithelium hypertrophy and bronchio-alveolar pneumonia and increased mucus-secreting neck cells in the stomach were shown in both animals dying before the end of the treatment period.

2. Body weight, food and water consumption, ophthalmoscopy and estrous cycles

Mean body weight is illustrated in Fig. 2. During the first four weeks of the treatment period the body weight gain and the food consumption of treated animals was similar to those of the control animals. During weeks 5 to 8 of treatment the body weight gain and the food consumption of males receiving 1200 mg/kg/day was decreased. Water consumption was slightly increased for males receiving 1200 mg/kg/day during weeks 1 to 4 of treatment.

Ophthalmoscopy examination during week 4 of treatment did not reveal any findings considered to be related to treatment with trientine-2HCl. There was no evidence of any dosage- or treatment-related effects on estrous cycles.

3. Clinical examinations

Hematological and blood chemical values and urinalysis are summarized in Tables 2 to 4. In hematological examinations, small, statistically significant increases were seen on both occasions of examination in respect to packed cell volume, hemoglobin concentration and erythrocyte count in males treated at 1200 mg/kg/day when compared with contemporary controls. A number of other statistically significant inter-group differences were seen, such as increased activated partial thromboplastin times and increased lymphocyte and platelet numbers. These probably represent chance variations but could reflect minor effects of treatment.

Blood chemistry investigations revealed some

Fig. 2. Mean body weight of rats in 4- or 8-week toxicity study of trientine-2HCl.

Table 2. Summary of hematological values in rats in 4- or 8-week toxicity study of trientine-2HCl.

Sex:		Males					Females				
Dosage (mg/kg/day):		0	100	350	1200		0	100	350	1200	
[During week 4 of treatment]											
(Number of animals)	(9) #	(10)	(10)	(10)	(10)		(10)	(10)	(10)	(10)	
PCV	42 ± 1	43 ± 1*	43 ± 1**	43 ± 1**	43 ± 1**		43 ± 0	42 ± 1	43 ± 1	44 ± 1*	
Hb	14.7 ± 0.3	15.1 ± 0.3**	15.1 ± 0.2**	15.2 ± 0.4***	15.2 ± 0.4***		15.4 ± 0.2	15.0 ± 0.3**	15.4 ± 0.4	15.7 ± 0.3	
RBC	7.92 ± 0.20	8.13 ± 0.18*	8.19 ± 0.19**	8.23 ± 0.18***	8.23 ± 0.18***		8.37 ± 0.09	8.16 ± 0.15**	8.36 ± 0.20	8.47 ± 0.21	
WBC - Total	8.2 ± 1.5	7.3 ± 0.8	7.5 ± 0.8	8.7 ± 1.2	8.7 ± 1.2		7.9 ± 0.9	7.6 ± 1.3	7.8 ± 1.1	8.4 ± 1.0	
- N	3.2 ± 1.0	2.4 ± 0.8*	2.4 ± 0.6*	2.9 ± 0.5	2.9 ± 0.5		2.1 ± 0.7	2.4 ± 0.8	2.3 ± 0.6	2.1 ± 0.6	
- L	4.7 ± 0.6	4.6 ± 0.4	4.8 ± 0.3	5.3 ± 1.2	5.3 ± 1.2		5.5 ± 0.3	4.8 ± 0.6*	5.2 ± 0.8	6.0 ± 0.8	
Platelets	859 ± 142	945 ± 54	951 ± 79*	886 ± 93	886 ± 93		794 ± 83	861 ± 42*	877 ± 30**	855 ± 60*	
PT	11.7 ± 0.5##	12.4 ± 0.3**	12.3 ± 0.4**	12.2 ± 0.5*	12.2 ± 0.5*		12.5 ± 0.5	13.0 ± 0.5	12.9 ± 0.6	12.7 ± 0.4	
APTT	17.2 ± 2.6##	17.1 ± 1.7	17.5 ± 1.7	20.8 ± 1.5***	20.8 ± 1.5***		16.5 ± 1.4	16.5 ± 1.5	17.1 ± 0.8	17.4 ± 1.7	
[During week 8 of treatment]											
(Number of animals)	(5)	(5)	(5)	(4)	(5)		(5)	(5)	(5)	(5)	
PCV	42 ± 1	43 ± 1	43 ± 2	44 ± 1*	42 ± 1		42 ± 1	42 ± 1	42 ± 2	42 ± 2	
Hb	14.5 ± 0.4	15.2 ± 0.3*	14.9 ± 0.6	15.5 ± 0.5**	15.1 ± 0.3		15.1 ± 0.3	14.9 ± 0.3	15.1 ± 0.6	14.9 ± 0.6	
RBC	8.22 ± 0.27	8.59 ± 0.23	8.35 ± 0.44	8.70 ± 0.26*	8.37 ± 0.19		8.37 ± 0.19	8.19 ± 0.26	8.28 ± 0.28	8.24 ± 0.41	
WBC - Total	8.7 ± 0.9	9.2 ± 0.6	7.9 ± 0.6**	9.6 ± 1.2	7.0 ± 1.4		7.0 ± 1.4	7.3 ± 1.1	7.9 ± 1.2	8.6 ± 1.3	
- N	4.0 ± 0.6	3.3 ± 0.4	2.7 ± 0.6	3.9 ± 0.5	1.9 ± 0.7		1.9 ± 0.7	2.5 ± 0.7	2.7 ± 0.9	2.3 ± 0.7	
- L	4.3 ± 1.0	5.5 ± 0.4*	4.8 ± 1.0	5.2 ± 0.7	4.8 ± 0.8		4.8 ± 0.8	4.4 ± 0.6	4.9 ± 0.4	5.8 ± 0.6*	
Platelets	739 ± 32	749 ± 34	809 ± 41**	805 ± 39*	797 ± 69		797 ± 69	812 ± 30	820 ± 43	871 ± 60*	
PT	13.1 ± 0.9	12.8 ± 0.5	13.5 ± 0.5	13.5 ± 0.3	13.7 ± 0.4		13.7 ± 0.4	14.1 ± 0.5	13.5 ± 0.5	13.5 ± 0.7	
APTT	20.6 ± 2.6	19.7 ± 1.3	19.8 ± 1.8	22.4 ± 2.5	18.4 ± 0.5		18.4 ± 0.5	18.5 ± 1.3	19.0 ± 1.4	19.5 ± 1.1	

Each value represents the Mean ± SD.

Significant when compared with control : * p<0.05; ** p<0.01; *** p<0.001.

: One sample was not available due to clotting.

: Number of animals : 10

Repeated dose toxicity studies of trientine-2HCl in F-344 rats.

Table 3. Summary of blood chemical values in rats in 4- or 8-week toxicity study of trientine-2HCl.

Sex:		Males				Females			
Dosage (mg/kg/day)		0	100	350	1200	0	100	350	1200
[During week 4 of treatment]									
(Number of animals)		(10)	(10)	(10)	(10)	(10)	(10)	(10)	(10)
ALP	iu/L	234 ± 26	241 ± 23	210 ± 24*	130 ± 20***	176 ± 22	187 ± 31	145 ± 14**	109 ± 27***
ALT	iu/L	56 ± 7	62 ± 5*	58 ± 6	69 ± 7***	50 ± 4	48 ± 5	48 ± 6	60 ± 6***
AST	iu/L	90 ± 8	100 ± 26	98 ± 13	97 ± 10	106 ± 17	101 ± 9	88 ± 6**	97 ± 10
Urea	mg/dL	36 ± 3	34 ± 2	33 ± 3**	35 ± 3	37 ± 4	38 ± 4	35 ± 5	36 ± 3
Gluc	mg/dL	144 ± 14	133 ± 6*	133 ± 10*	143 ± 12	129 ± 13	136 ± 10	128 ± 12	131 ± 16
Trig	mg/dL	113 ± 24	140 ± 24*	135 ± 29	150 ± 39**	68 ± 14	51 ± 18	65 ± 29	82 ± 20
Chol	mg/dL	75 ± 7	78 ± 5	85 ± 14*	85 ± 6*	100 ± 7	94 ± 6	93 ± 8*	89 ± 5***
TP	g/dL	5.9 ± 0.2	6.4 ± 0.2***	6.2 ± 0.2*	5.9 ± 0.3	6.0 ± 0.3	5.8 ± 0.1	5.8 ± 0.2	5.6 ± 0.2**
Na	mmol/L	142 ± 1	145 ± 1*	141 ± 5	144 ± 4	140 ± 1	142 ± 2**	138 ± 1*	140 ± 1
K	mmol/L	3.6 ± 0.2	3.5 ± 0.2	3.5 ± 0.2	3.6 ± 0.2	3.5 ± 0.2	3.5 ± 0.2	3.3 ± 0.2**	3.6 ± 0.2
Cl	mmol/L	103 ± 1	105 ± 1	102 ± 3	103 ± 2	103 ± 1	105 ± 1***	102 ± 1**	102 ± 1
[During week 8 of treatment]									
(Number of animals)		(5)	(5)	(5)	(4)	(5)	(5)	(5)	(5)
ALP	iu/L	148 ± 13	141 ± 8	115 ± 4***	67 ± 3***	122 ± 16	106 ± 11*	91 ± 13***	50 ± 2***
ALT	iu/L	50 ± 5	50 ± 6	51 ± 6	79 ± 8***	47 ± 5	39 ± 2	46 ± 4	68 ± 11***
AST	iu/L	97 ± 9	89 ± 6	80 ± 8**	98 ± 7	97 ± 6	83 ± 6**	82 ± 6**	96 ± 7
Urea	mg/dL	35 ± 3	37 ± 4	38 ± 2	43 ± 1***	37 ± 5	44 ± 3**	41 ± 1	47 ± 4***
Gluc	mg/dL	163 ± 18	152 ± 17	146 ± 19	131 ± 16*	156 ± 18	163 ± 11	142 ± 10	123 ± 13**
Trig	mg/dL	117 ± 25	138 ± 36	118 ± 28	198 ± 29***	54 ± 7	59 ± 8	81 ± 28*	101 ± 22***
Chol	mg/dL	70 ± 4	70 ± 6	68 ± 5	83 ± 5**	92 ± 7	79 ± 5*	86 ± 9	87 ± 12
TP	g/dL	5.7 ± 0.3	6.2 ± 0.3*	5.9 ± 0.3	5.7 ± 0.3	5.6 ± 0.3	5.7 ± 0.1	5.6 ± 0.4	5.2 ± 0.3*
Na	mmol/L	136 ± 5	138 ± 2	137 ± 1	138 ± 1	139 ± 2	140 ± 2	138 ± 4	138 ± 2
K	mmol/L	3.7 ± 0.2	3.3 ± 0.1**	3.2 ± 0.1***	3.7 ± 0.3	3.7 ± 0.2	3.3 ± 0.2*	3.4 ± 0.1*	3.2 ± 0.3**
Cl	mmol/L	103 ± 1	101 ± 1***	102 ± 1*	102 ± 1**	104 ± 1	106 ± 1	105 ± 3	104 ± 1

Each value represents the Mean ± SD.

Significant when compared with control : *p<0.05; **p<0.01; ***p<0.001.

Table 4. Urinalysis for rats in 4- or 8-week toxicity study of trientine-2HCl.

Sex:	Males						Females					
Dosage (mg/kg/day):	0	100	350	1200	0	100	350	1200	0	100	350	1200
[during week 4 of treatment ; Interval 2]												
(Number of animals)	(10)	(10)	(10)	(10)	(10)	(10)	(10)	(10)	(10)	(10)	(10)	(9)#
Volume mL	3.5 ± 0.5	4.0 ± 0.5	3.0 ± 0.5	3.0 ± 1.0	2.0 ± 0.5	2.5 ± 0.5	2.0 ± 1.0	2.0 ± 0.5	2.0 ± 0.5	2.5 ± 0.5	2.0 ± 1.0	2.0 ± 0.5
pH	6.4 ± 0.3	6.3 ± 0.2	6.1 ± 0.3**	5.4 ± 0.1***	6.1 ± 0.2	6.0 ± 0.1	5.9 ± 0.3*	5.3 ± 0.1***	5.3 ± 0.1***	6.0 ± 0.1	5.9 ± 0.3*	5.3 ± 0.1***
SG ×10 ⁻³	1036 ± 6	1035 ± 3	1043 ± 9 *	1047 ± 9 **	1051 ± 11	1050 ± 7	1062 ± 24	1070 ± 12**	1070 ± 12**	1050 ± 7	1062 ± 24	1070 ± 12**
Na mmol	0.28 ± 0.09	0.33 ± 0.08	0.34 ± 0.07	0.50 ± 0.11***	0.23 ± 0.07	0.26 ± 0.08	0.26 ± 0.11	0.36 ± 0.08**	0.36 ± 0.08**	0.26 ± 0.08	0.26 ± 0.11	0.36 ± 0.08**
K mmol	0.73 ± 0.16	0.76 ± 0.10	0.67 ± 0.11	0.54 ± 0.10**	0.57 ± 0.18	0.53 ± 0.08	0.43 ± 0.19*	0.46 ± 0.08	0.46 ± 0.08	0.53 ± 0.08	0.43 ± 0.19*	0.46 ± 0.08
Cl mmol	0.43 ± 0.10	0.49 ± 0.08	0.55 ± 0.09	1.24 ± 0.28***	0.34 ± 0.09	0.37 ± 0.07	0.38 ± 0.16	0.92 ± 0.34***	0.92 ± 0.34***	0.37 ± 0.07	0.38 ± 0.16	0.92 ± 0.34***
[during week 8 of treatment ; Interval 1]												
(Number of animals)	(5)	(5)	(5)	(5)	(5)	(5)	(5)	(5)	(5)	(5)	(5)	(5)
Volume mL	5.0 ± 0.5	6.0 ± 1.0	5.0 ± 0.5	2.0 ± 0.5***	4.5 ± 2.0	4.5 ± 0.5	4.5 ± 1.0	1.5 ± 0.5***	1.5 ± 0.5***	4.5 ± 0.5	4.5 ± 1.0	1.5 ± 0.5***
Na mmol	0.14 ± 0.04	0.31 ± 0.05 *	0.36 ± 0.05*	0.53 ± 0.24***	0.18 ± 0.07	0.14 ± 0.03	0.22 ± 0.10	0.23 ± 0.10	0.23 ± 0.10	0.14 ± 0.03	0.22 ± 0.10	0.23 ± 0.10
K mmol	0.23 ± 0.06	0.41 ± 0.11**	0.51 ± 0.08***	0.45 ± 0.06***	0.23 ± 0.06	0.28 ± 0.04	0.31 ± 0.08	0.39 ± 0.10**	0.39 ± 0.10**	0.28 ± 0.04	0.31 ± 0.08	0.39 ± 0.10**
Cl mmol	0.17 ± 0.04	0.47 ± 0.11***	0.81 ± 0.06***	1.00 ± 0.19***	0.17 ± 0.06	0.24 ± 0.02	0.52 ± 0.16 **	0.65 ± 0.22***	0.65 ± 0.22***	0.24 ± 0.02	0.52 ± 0.16 **	0.65 ± 0.22***
[during week 8 of treatment ; Interval 2]												
(Number of animals)	(5)	(5)	(5)	(5)	(5)	(5)	(5)	(5)	(5)	(5)	(5)	(5)
Volume mL	8.5 ± 2.5	9.0 ± 3.5	6.5 ± 1.5	8.5 ± 2.5	7.0 ± 2.0	7.0 ± 2.5	6.0 ± 1.5	6.5 ± 3.0	6.5 ± 3.0	7.0 ± 2.5	6.0 ± 1.5	6.5 ± 3.0
pH	6.5 ± 0.1	7.0 ± 0.3***	6.4 ± 0.1	5.6 ± 0.1***	6.3 ± 0.2	6.8 ± 0.2*	6.5 ± 0.4	5.8 ± 0.3*	5.8 ± 0.3*	6.8 ± 0.2*	6.5 ± 0.4	5.8 ± 0.3*
SG ×10 ⁻³	1021 ± 6	1021 ± 5	1028 ± 6	1026 ± 8	1021 ± 5	1022 ± 4	1022 ± 5	1024 ± 8	1024 ± 8	1022 ± 4	1022 ± 5	1024 ± 8
Na mmol	0.15 ± 0.04	0.24 ± 0.03	0.29 ± 0.05**	0.41 ± 0.12***	0.14 ± 0.05	0.22 ± 0.04	0.21 ± 0.08	0.30 ± 0.12**	0.30 ± 0.12**	0.22 ± 0.04	0.21 ± 0.08	0.30 ± 0.12**
K mmol	0.70 ± 0.08	0.76 ± 0.13	0.69 ± 0.12	0.58 ± 0.07	0.38 ± 0.14	0.46 ± 0.06	0.40 ± 0.20	0.40 ± 0.14	0.40 ± 0.14	0.46 ± 0.06	0.40 ± 0.20	0.40 ± 0.14
Cl mmol	0.30 ± 0.07	0.36 ± 0.07	0.49 ± 0.08**	1.34 ± 0.16***	0.21 ± 0.09	0.28 ± 0.06	0.30 ± 0.08	0.84 ± 0.39 ***	0.84 ± 0.39 ***	0.28 ± 0.06	0.30 ± 0.08	0.84 ± 0.39 ***

Interval 1 : Urine collected immediately after dosing until approximately 5 p.m.

Interval 2 : Urine collected overnight from approximately 5 p.m. to approximately 9 a.m.

Each value represents the Mean ± SD.

Significant when compared with control: *p<0.05; **p<0.01; ***p<0.001.

: One sample was unsuitable for analysis due to contamination.

differences statistically significant from the control values which were consistently seen and therefore attributed to treatment; these consisted of low alkaline phosphatase activity in animals treated at 350 or 1200 mg/kg/day and high alanine amino-transferase activity in rats treated at 1200 mg/kg/day. Other changes that were clearly attributable to treatment were confined to animals receiving the high dosage. These changes consisted of high triglyceride and urea concentrations and low glucose concentrations, high cholesterol and low total protein concentrations confined to males and females, respectively.

Urinalysis during week 4 of treatment revealed low urinary pH and a slightly high specific gravity in animals receiving 350 or 1200 mg/kg/day; and increased sodium and chloride and decreased potassium output in animals receiving 1200 mg/kg/day. Results of urinalysis performed during week 8 of treatment were similar to those during week 4 of treatment except that during Interval 1 there was a markedly decreased urinary output in animals receiving 1200 mg/kg/day and an increased potassium output in treated animals receiving 1200 mg/kg/day. There were no other changes in clinical examinations which could be associated with treatment.

4. Pathological examination

Organ weights and histopathological findings are summarized in Tables 5 and 6. Slightly increased relative kidney weights in animals receiving 1200 mg/kg/day were noted after 4 and/or 8 weeks of treatment. Slightly increased relative and/or absolute lung weights were noted in animals receiving 1200 mg/kg/day after 8 weeks of treatment. Other changes of organ weight were also confined to animals receiving the high dosage. These changes consisted of slightly increased relative liver weights for females after 4 weeks of treatment, and slightly decreased absolute and relative thymus weight and slightly increased relative adrenal weights for males after 8 weeks of treatment. There were no other changes in organ weights which could be associated with treatment.

Macropathological findings that were attributable to treatment were confined to animals receiving 1200 mg/kg/day. These findings consisted of areas of change in the lung of one female killed after 8 weeks of treatment, dark lung in one male that died during the treatment period and a thin appearance in one male killed after 8 weeks of treatment and in one male that died during the treatment period.

Histopathological changes considered to be related

to treatment were found mainly in the lungs and stomach. A high incidence of bronchiolar epithelium hypertrophy and broncho-alveolar pneumonia were found in the lungs of males and females receiving 1200 mg/kg/day, with a higher incidence in the males. In addition, there was a slightly higher than expected incidence of accumulation of alveolar macrophages in males receiving 1200 mg/kg/day. There were no similar changes observed in the lungs from animals receiving 100 or 350 mg/kg/day. In the stomach, submucosal acute inflammation within the glandular region was recorded for males that received 350 and 1200 mg/kg/day and in all treated female groups. For animals receiving 100 and 350 mg/kg/day, this finding was found only in animals killed after 4 weeks of treatment, with the exception of one male killed after 8 weeks that received 350 mg/kg/day. There was also an increased incidence of mucus-secreting neck cells of animals receiving 1200 mg/kg/day killed after 4 or 8 weeks of treatment. It is also considered to be treatment-related that cortical atrophy of the thymus was found in one male killed after 8 weeks of treatment and in the two males that died during the treatment period, and that lymphocytolysis of the mandibular lymph nodes was found in the two males that died during the treatment period.

26-week study

1. Signs and mortality

There were no signs that could be unequivocally associated with treatment except in the following animals which were found dead or killed *in extremis*. Two males receiving 600 mg/kg/day were found dead during weeks 13 or 23 of treatment. Two males, one each receiving 175 or 600 mg/kg/day, were killed *in extremis* during weeks 14 or 19 of treatment, respectively. One control male was killed *in extremis* during week 2 of the reversibility period. Of the animals killed *in extremis*, signs prior to dispatch included reduced body temperature, shallow or fast respiration, pallor and underactivity. At necropsy, dark lungs were recorded for the two high dosage males that died. All of the treated males that died, or were killed, showed perivascular edema, necrosis and regenerative hyperplasia of the terminal bronchioles, acute interstitial pneumonitis, bronchiolar hyperplasia and bronchiolar epithelium hypertrophy of the lungs. These deaths were considered to be treatment-related. The histopathological findings for the control male that was killed during the reversibility period included monocytic leukaemia, and this was considered to be the cause of death.

Table 5. Summary of organ weights in rats in 4- or 8-week toxicity study of trientine-2HCl.

Dosage (mg/kg/day):		Male				Female			
		0	100	350	1200	0	100	350	1200
[After 4 weeks of treatment]									
(Number of animals)		(5)	(5)	(5)	(5)	(5)	(5)	(5)	(5)
Body weight	g	185.9 ± 17.9	196.8 ± 10.4	188.8 ± 26.8	185.1 ± 16.7	139.7 ± 4.3	137.5 ± 5.4	141.0 ± 7.3	136.2 ± 4.1
Adrenals	mg	26 ± 2 ^{a)}	27 ± 5	28 ± 3	26 ± 3	32 ± 2	31 ± 2	37 ± 7	37 ± 6
	mg/100gBW	14.1 ± 1.8 ^{b)}	13.8 ± 2.5	14.9 ± 1.4	14.2 ± 2.4	22.7 ± 1.1	22.7 ± 2.4	26.0 ± 3.9	27.1 ± 3.7
Kidneys	g	1.24 ± 0.13	1.26 ± 0.09	1.23 ± 0.17	1.31 ± 0.07	0.97 ± 0.09	0.97 ± 0.03	1.01 ± 0.07	1.03 ± 0.04
	g/100gBW	0.669 ± 0.011	0.645 ± 0.022	0.653 ± 0.017	0.708 ± 0.035*	0.695 ± 0.044	0.705 ± 0.026	0.713 ± 0.026	0.753 ± 0.019*
Liver	g	7.4 ± 1.1	7.8 ± 0.9	7.8 ± 1.2	7.6 ± 1.0	5.0 ± 0.5	4.9 ± 0.1	5.1 ± 0.3	5.4 ± 0.4
	g/100gBW	3.96 ± 0.25	3.96 ± 0.25	4.13 ± 0.26	4.12 ± 0.29	3.56 ± 0.30	3.57 ± 0.16	3.63 ± 0.17	3.99 ± 0.23*
Lungs	g	0.88 ± 0.08	0.87 ± 0.06#	0.91 ± 0.07	0.90 ± 0.07	0.76 ± 0.10	0.73 ± 0.04	0.79 ± 0.15	0.70 ± 0.03
	g/100gBW	0.476 ± 0.024	0.448 ± 0.012#	0.488 ± 0.052	0.491 ± 0.061	0.545 ± 0.053	0.528 ± 0.019	0.560 ± 0.080	0.513 ± 0.019
Thymus	mg	330 ± 28	377 ± 52	315 ± 20	284 ± 27	305 ± 49	301 ± 36	293 ± 24	288 ± 45
	mg/100gBW	178.8 ± 20.2	192.1 ± 21.6	170.6 ± 32.7	153.7 ± 11.4	218.7 ± 36.6	219.1 ± 23.7	208.3 ± 21.7	211.2 ± 29.4
[After 8 weeks of treatment]									
(Number of animals)		(5)	(5)	(5)	(3)	(5)	(5)	(5)	(5)
Body weight	g	229.6 ± 14.6	242.9 ± 15.3	240.0 ± 13.8	200.0 ± 12.0*	149.1 ± 6.5	158.5 ± 4.2	151.8 ± 13.9	151.0 ± 7.1
Adrenals	mg	28 ± 6	32 ± 3	33 ± 3	32 ± 6	32 ± 7	34 ± 2	36 ± 5	37 ± 4
	mg/100gBW	12.0 ± 2.3	13.1 ± 0.7	13.8 ± 1.2	16.1 ± 2.7*	21.7 ± 4.4	21.3 ± 1.0	23.9 ± 2.9	24.4 ± 3.2
Kidneys	g	1.34 ± 0.12	1.43 ± 0.12	1.44 ± 0.06	1.29 ± 0.08	1.01 ± 0.06	1.03 ± 0.06	1.00 ± 0.07	1.04 ± 0.06
	g/100gBW	0.585 ± 0.023	0.589 ± 0.024	0.599 ± 0.026	0.645 ± 0.011**	0.679 ± 0.021	0.650 ± 0.020	0.662 ± 0.023	0.686 ± 0.028
Liver	g	8.0 ± 0.8	8.6 ± 1.0	8.9 ± 0.6	7.1 ± 0.2	4.7 ± 0.3	4.7 ± 0.7	4.9 ± 0.4	5.1 ± 0.4
	g/100gBW	3.48 ± 0.22	3.55 ± 0.22	3.70 ± 0.16	3.55 ± 0.14	3.18 ± 0.12	2.97 ± 0.39	3.26 ± 0.10	3.35 ± 0.25
Lungs	g	0.91 ± 0.07	1.01 ± 0.14	1.01 ± 0.09	1.18 ± 0.27*	0.72 ± 0.04	0.78 ± 0.08	0.78 ± 0.05	0.79 ± 0.05
	g/100gBW	0.395 ± 0.023	0.417 ± 0.049	0.420 ± 0.038	0.586 ± 0.120**	0.481 ± 0.018	0.493 ± 0.042	0.517 ± 0.028	0.525 ± 0.049
Thymus	mg	313 ± 46	320 ± 38	282 ± 44	211 ± 17**	245 ± 23	259 ± 63	227 ± 27	226 ± 65
	mg/100gBW	136.3 ± 16.1	131.7 ± 9.5	117.4 ± 17.7	105.8 ± 7.6*	164.6 ± 17.5	163.2 ± 37.0	150.3 ± 20.2	148.6 ± 39.3

Each value represents the Mean ± SD

a) : Upper column indicates absolute organ weight.

b) : Lower column indicates organ weight relative to body weight

Significant when compared with control : * p<0.05; ** p<0.01.

: Number of animals : 4

Repeated dose toxicity studies of trientine-2HCl in F-344 rats.

Table 6. Summary of histopathological findings for rats in 4- or 8-week toxicity study of trientine-2HCl.

Sex:	Males				Females			
	0	100	350	1200	0	100	350	1200
Dosage (mg/kg/day):								
Number of animals:	10	10	10	10(2)	10	10	10	10
Kidneys L&R	10	0	0	10(2)	10	0	0	10
Medullary mineralization	8			7(1)	8			7
Papillary mineralization	6			6(1)	6			5
Basophilic cortical tubules	4			0	1			1
Cortico-medullary mineralization	0			0	6			2
Cortical mineralization	0			0	1			0
Chronic interstitial nephritis	0			0	0			1
Lymph nodes Mandibular	10	0	0	10(2)	10	0	0	10
Plasmacytosis	4			2	4			2
Sinus histiocytosis	1			0	0			0
Follicular hyperplasia	5			6(1)	3			3
Lymphocytolysis	0			(2)	0			0
Liver x 2	10	0	0	10(2)	10	1	0	10
Median lobe anomaly	0			0	0	1		1
Chronic inflammatory cells within the portal area	0			2(1)	0	0		0
Lungs x 2	10	10	10	10(2)	10	10	10	10
Accumulation(s) of alveolar macrophages	0	0	0	2	0	0	0	0
Bronchiolar epithelium hypertrophy	0	0	0	7(2)**	0	0	0	3
Broncho-alveolar pneumonia	0	0	0	7(2)**	0	0	0	3
Goblet cell hyperplasia	0	0	0	(2)	0	0	0	0
Bronchiolization of alveolar wall	0	0	0	(1)	0	0	0	2
Alveolar hyperplasia	0	0	0	1	0	0	0	0
Interstitial pneumonitis	2	4	5	1	4	3	4	2
Stomach x 2	10	10	10	10(2)	10	10	10	10
Submucosal acute inflammation within the glandular region	0	0	3	8(1)***	0	3	2	8***
Increased mucus-secreting neck cells	0	0	1	10(2)***	0	0	0	9***
Keratinized region: Hyperkeratosis and acanthosis	0	0	0	0	0	0	0	1
Thymus	10	0	0	10(2)	10	0	0	10
Cortical atrophy	0			3(2)	0	0	0	0
Uterus	0	0	0	0	10	0	1	10
Dilated					2		1	2

Significant when compared with control : **p<0.01; *** p<0.001.

The figures in parentheses show the number of rats found dead or killed in *extremis*.

2. Body weight, food and water consumption and ophthalmoscopy

Mean body weight is illustrated in Fig. 3. The body weight gain of animals receiving 600 mg/kg/day was slightly decreased; this was most marked in the males. Body weight gain during the reversibility period was similar between the control group and animals previously receiving 600 mg/kg/day.

The overall food intake, during both the treatment and reversibility periods, was similar between the control and treated groups. Water consumption measurements, performed over three days, showed that the water intake of animals receiving 600 mg/kg/day was slightly increased during week 1 of treatment (male; 18.2 ± 1.07 vs 16.1 ± 0.96 mL/rat/day, female; 15.2 ± 0.66 vs 13.5 ± 0.84 mL/rat/day) with males at this dosage similarly affected during week 6 of treatment (18.2 ± 0.78 vs 16.5 ± 0.89 mL/rat/day). Water consumption, when measured during urine collection, was unaffected by treatment.

There were no ocular changes considered to be related to treatment.

3. Clinical examinations

Hematological, blood chemical and urinalysis data are summarized in Tables 7-1 to 9-2. Hematological examinations at the end of both the treatment and reversibility periods showed several small, statistically significant changes at the high dosage; however, these changes were minor and the values were within the

normal range expected.

Blood chemistry investigations at the end of the treatment period revealed low alkaline phosphatase activities in animals receiving 600 mg/kg/day and males receiving 175 mg/kg/day. Animals receiving 600 mg/kg/day also showed low concentrations of cholesterol, total protein, creatinine (male only) and urea (female only). Low cholesterol concentrations were also noted in males receiving 175 mg/kg/day. There was evidence of electrolyte disturbance in all treated groups, although these changes often lacked dosage relationship or were confined to one sex.

Results of urinalysis during Interval 1 at the end of the treatment period were similar to those found during the 4- or 8-week study. On both sampling occasions, decreased urinary output and low pH, and increased electrolyte outputs (sodium, potassium and chloride) were evident in animals receiving 600 mg/kg/day. Low pH and increased chloride output were also evident, at both sampling occasions, in animals receiving 175 mg/kg/day. During Interval 2 on both sampling occasions, slightly low pH, decreased potassium output and increased chloride output were noted in animals receiving 600 mg/kg/day. All these changes seen in the blood chemistry examination and urinalysis at the end of the treatment period were not evident or clearly reduced after the reversibility period. There were no other changes in clinical examinations which could be associated with treatment.

Fig. 3. Mean body weight of rats in 26-week toxicity study of trientine-2HCl.

Table 7-1. Summary of hematological values in rats at the end of treatment period in 26-week toxicity study of trientine-2HCl.

Sex:		Males				Females			
Dosage (mg/kg/day):		0	50	175	600	0	50	175	600
(Number of animals)		(16)	(12)	(8)	(15)	(19)	(10)	(11)	(20)
PCV	%	44 ± 1	44 ± 1	43 ± 1	45 ± 1**	44 ± 1	44 ± 1	44 ± 1	45 ± 1*
Hb	g/dL	15.2 ± 0.2	15.3 ± 0.3	15.2 ± 0.4	15.6 ± 0.5**	15.7 ± 0.3	15.5 ± 0.3	15.5 ± 0.4	16.0 ± 0.5**
RBC	× 10 ⁶ /mm ³	9.15 ± 0.18	9.19 ± 0.10	9.01 ± 0.22	9.13 ± 0.28	8.56 ± 0.23	8.59 ± 0.19	8.51 ± 0.18	8.72 ± 0.26*
WBC	× 10 ³ /mm ³	6.8 ± 1.3	6.4 ± 1.2	6.4 ± 1.1	7.4 ± 1.7	5.6 ± 0.8	4.7 ± 0.5*	6.1 ± 0.9	6.1 ± 1.1
Platelets	× 10 ³ /mm ³	755 ± 70	747 ± 43	761 ± 32	777 ± 35	713 ± 97	715 ± 44	750 ± 41	775 ± 35**
(Number of animals)		(20)	(12)	(11)	(17)	(19)	(12)	(12)	(20)
PT	secs	12.6 ± 0.7	12.9 ± 0.3	12.9 ± 0.9	13.3 ± 0.5***	12.9 ± 1.0	12.7 ± 1.1	12.9 ± 0.5	12.9 ± 0.9
APTT	secs	19.3 ± 2.0	19.4 ± 0.7	19.4 ± 1.4	21.1 ± 1.0***	18.6 ± 1.6	17.4 ± 1.7*	17.2 ± 1.0*	18.4 ± 1.6

Each value represents the Mean ± SD.

Significant when compared with control: * p<0.05; ** p<0.01; *** p<0.001.

Table 7-2. Summary of haematological values in rats at the end of reversibility period in 26-week toxicity study of trientine-2HCl.

Sex:		Males				Females			
Dosage (mg/kg/day):		0	50	175	600	0	50	175	600
(Number of animals)		(7)	(7)	(8)	(6)##	(8)	(8)	(6)##	(6)##
PCV	%	44 ± 1	44 ± 1	46 ± 1**	46 ± 1**	44 ± 1	44 ± 1	44 ± 1	44 ± 1
Hb	g/dL	14.9 ± 0.3	14.9 ± 0.3	15.5 ± 0.2**	15.5 ± 0.2**	15.5 ± 0.3	15.5 ± 0.3	15.4 ± 0.3	15.4 ± 0.3
RBC	× 10 ⁶ /mm ³	9.08 ± 0.21	9.08 ± 0.21	9.34 ± 0.15*	9.34 ± 0.15*	8.49 ± 0.22	8.49 ± 0.22	8.42 ± 0.15	8.42 ± 0.15
WBC	× 10 ³ /mm ³	5.9 ± 0.5	5.9 ± 0.5	5.8 ± 0.7	5.8 ± 0.7	3.2 ± 0.5	3.2 ± 0.5	3.4 ± 1.0	3.4 ± 1.0
Platelets	× 10 ³ /mm ³	738 ± 26	738 ± 26	732 ± 63	732 ± 63	707 ± 43	707 ± 43	680 ± 36	680 ± 36
(Number of animals)		(7)	(7)	(7)	(7)	(8)	(8)	(8)	(8)
PT	secs	12.6 ± 0.4	12.6 ± 0.4	13.4 ± 0.8*	13.4 ± 0.8*	12.3 ± 0.3	12.3 ± 0.3	12.3 ± 0.3	12.3 ± 0.3
APTT	secs	20.3 ± 0.4	20.3 ± 0.4	20.2 ± 1.2	20.2 ± 1.2	16.9 ± 0.8	16.9 ± 0.8	16.4 ± 1.2	16.4 ± 1.2

Each value represents the Mean ± SD.

Significant when compared with control: *p<0.05; **p<0.01.

: One sample was not available due to clotting.

: Two samples were not available due to clotting.

Table 8-1. Summary of blood chemical values in rats at the end of treatment period in 26-week toxicity study of trientine-2HCl.

Sex:	Dosage (mg/kg/day):	Males				Females			
		0		175		50		175	
		20	12	11	17	12	12	12	20
ALP	iu/L	86 ± 12	87 ± 7	78 ± 6 *	59 ± 6***	71 ± 14	57 ± 10**	68 ± 12	48 ± 8***
ALT	iu/L	96 ± 104	77 ± 13	82 ± 18	75 ± 6	118 ± 49	114 ± 55	119 ± 53	131 ± 79
AST	iu/L	106 ± 50	105 ± 18	105 ± 30	87 ± 13	160 ± 59	191 ± 88	158 ± 59	167 ± 91
Urea	mg/dL	36 ± 3	36 ± 2	35 ± 2	35 ± 3	36 ± 4	35 ± 4	39 ± 3	33 ± 3**
Crea	mg/dL	0.71 ± 0.03	0.72 ± 0.03	0.70 ± 0.03	0.67 ± 0.03***	0.53 ± 0.04	0.58 ± 0.06**	0.63 ± 0.03***	0.54 ± 0.03
Trig	mg/dL	186 ± 29	194 ± 26	175 ± 27	168 ± 37	65 ± 12	74 ± 14	81 ± 23**	72 ± 11
Chol	mg/dL	92 ± 11	88 ± 9	83 ± 7 *	81 ± 10***	125 ± 12	122 ± 12	117 ± 16	105 ± 9***
TP	g/dL	6.8 ± 0.2	6.8 ± 0.2	6.7 ± 0.1	6.3 ± 0.1***	6.8 ± 0.2	6.9 ± 0.2	6.8 ± 0.4#	6.5 ± 0.2***
Na	mmol/L	137 ± 1	139 ± 1***	139 ± 1***	138 ± 1	137 ± 1	138 ± 1***	139 ± 2***	137 ± 1
K	mmol/L	3.4 ± 0.1	3.4 ± 0.2	3.6 ± 0.2***	3.4 ± 0.2	3.4 ± 0.3	3.5 ± 0.3	3.3 ± 0.2	3.3 ± 0.3
Cl	mmol/L	101 ± 1	103 ± 1***	106 ± 2***	104 ± 1***	104 ± 1	105 ± 1**	106 ± 1***##	104 ± 1
Ca	mmol/L	2.76 ± 0.04	2.74 ± 0.04	2.67 ± 0.03***	2.70 ± 0.05***	2.76 ± 0.04	2.72 ± 0.09	2.72 ± 0.08	2.74 ± 0.05
P	mmol/L	2.19 ± 0.13	2.18 ± 0.12	2.24 ± 0.14	2.25 ± 0.12	2.02 ± 0.18	1.80 ± 0.25**	1.88 ± 0.21 *	1.84 ± 0.16**

Each value represents the Mean ± SD.

Significant when compared with control: * p<0.05; ** p<0.01; *** p<0.001.

: Number of animals ; 11

: Number of animals ; 10

4. Metal analysis

The results of metal analysis are shown in Tables 10-1 and 10-2. Low plasma copper concentrations were found in males receiving 600 mg/kg/day. A high urinary copper concentration was found in all treated groups, with dosage relationship being evident. Slightly low copper concentrations were found in the liver of treated animals receiving 600 or 175 mg/kg/day. Females receiving 600 or 175 mg/kg/day showed low plasma iron and slightly low plasma zinc concentrations. Low urinary iron and high urinary zinc concentrations were noted in all treated male groups with a dosage relationship being evident. High urinary zinc concentrations were seen in females receiving 600 mg/kg/day and high urinary iron concentrations in females receiving 600 or 175 mg/kg/day. None of these changes were evident at the end of the reversibility period. There were no changes in metal concentrations in the kidneys considered to be related to treatment.

5. Pathological examination

Organ weights and histopathological findings are summarized in Tables 11-1 to 12-2. Relative kidney weights were slightly increased in males receiving 600 mg/kg/day. This was still evident at the end of the reversibility period. Slightly decreased absolute and relative spleen weights were noted in males receiving 600 mg/kg/day, although in the absence of any change

in the females or any histopathological correlate, this was considered to be of no toxicological significance. All other organ weight changes were either associated with the difference in body weight at necropsy, confined to one sex or lacked dosage relationship and were therefore considered to be fortuitous.

There was an increased incidence of pale areas of the lungs in animals receiving 600 mg/kg/day. This was still evident at the end of the reversibility period.

Treatment-related histopathological findings were seen in the lungs, with males more affected than females. There was a dosage-related incidence and severity of focal chronic interstitial pneumonitis which was frequently accompanied by fibrosis of the alveolar walls (Photo. 1). The lesion was present in the lungs of all animals receiving 600 mg/kg/day and in the majority of animals receiving 175 mg/kg/day. There were no lung changes in females receiving 50 mg/kg/day, but an increased number of the males from this group exhibited minimal pneumonitis, four of them showing evidence of alveolar fibrosis. One control male also showed minimal focal interstitial pneumonitis, but this was not associated with any fibrosis. The interstitial pneumonitis in the treated animals was characterized by focal areas of alveolar collapse with varying degrees of fibrosis. The focal interstitial lesions were generally localized around bronchioles which were frequently increased in number (bronchiolar hyperplasia) and

Table 8-2. Summary of blood chemical values in rats at the end of reversibility period in 26-week toxicity study of trientine-2HCl.

Sex:		Males		Females	
Dosage (mg/kg/day):		0	600	0	600
Number of animals:		7	7	8	8
ALP	iu/L	96 ± 6	98 ± 15	66 ± 15	75 ± 16
ALT	iu/L	151 ± 33	208 ± 117	120 ± 38	96 ± 35
AST	iu/L	176 ± 45	237 ± 110	153 ± 37	143 ± 52
Urea	mg/dL	37 ± 3	39 ± 4	46 ± 3	48 ± 4
Crea	mg/dL	0.67 ± 0.02	0.67 ± 0.02	0.69 ± 0.03	0.69 ± 0.02
Trig	mg/dL	132 ± 15	116 ± 19	103 ± 19	93 ± 19
Chol	mg/dL	106 ± 5	105 ± 16	145 ± 12	139 ± 17
TP	g/dL	6.7 ± 0.1	6.9 ± 0.2	7.3 ± 0.1	7.1 ± 0.1
Na	mmol/L	138 ± 1	140 ± 1 **	139 ± 1	139 ± 1
K	mmol/L	3.8 ± 0.2	3.9 ± 0.2	3.5 ± 0.2	3.5 ± 0.3
Cl	mmol/L	101 ± 1	103 ± 0***	104 ± 1	105 ± 1 **
Ca	mmol/L	2.78 ± 0.03	2.82 ± 0.04	2.84 ± 0.04	2.78 ± 0.06 *
P	mmol/L	2.08 ± 0.15	2.01 ± 0.13	1.49 ± 0.14	1.27 ± 0.26

Each value represents the Mean ± SD.

Significant when compared with control: * p<0.05; ** p<0.01; *** p<0.001.

Table 9-1. Urinalysis for rats at the end of treatment period in 26-week toxicity study of trientine-2HCl.

Sex:		Males				Females			
Dosage (mg/kg/day):	0	50	175	600	0	50	175	600	
Number of animals:	20	12	11	17	20	12	12	20	
[Interval 1]									
Volume	mL	5.4 ± 1.5	5.3 ± 1.2	4.9 ± 1.3	3.8 ± 1.0***	2.8 ± 1.1	2.1 ± 0.6*	2.5 ± 0.8	
pH		7.5 ± 0.3	7.5 ± 0.4	6.8 ± 0.3***	6.0 ± 0.2***	7.3 ± 0.5	7.0 ± 0.4*	5.8 ± 0.1***	
Na	mmol	0.24 ± 0.08	0.24 ± 0.08	0.31 ± 0.07*	0.40 ± 0.11***	0.18 ± 0.08	0.16 ± 0.06	0.34 ± 0.09***	
K	mmol	0.45 ± 0.12	0.43 ± 0.07	0.52 ± 0.08	0.59 ± 0.10***	0.30 ± 0.10	0.23 ± 0.05*	0.36 ± 0.08*	
Cl	mmol	0.27 ± 0.08	0.28 ± 0.07	0.67 ± 0.10***	1.20 ± 0.24***	0.21 ± 0.08	0.21 ± 0.08	0.85 ± 0.18***	
[Interval 2]									
Volume	mL	5.1 ± 1.5	4.8 ± 1.8	4.7 ± 1.0	5.5 ± 1.4	4.5 ± 3.0	2.4 ± 1.2*	3.9 ± 2.0	
pH		6.5 ± 0.3	6.6 ± 0.3	6.5 ± 0.3	6.3 ± 0.2**	6.5 ± 0.4	6.4 ± 0.6	6.3 ± 0.4	
SG	× 10 ⁻³	1035 ± 7	1033 ± 6	1034 ± 6	1028 ± 5***	1032 ± 13	1044 ± 12*	1028 ± 11	
Na	mmol	0.35 ± 0.08	0.26 ± 0.07**	0.29 ± 0.12	0.30 ± 0.10	0.18 ± 0.06	0.18 ± 0.05	0.13 ± 0.05**	
K	mmol	0.70 ± 0.13	0.59 ± 0.11*	0.63 ± 0.10	0.52 ± 0.11***	0.43 ± 0.13	0.36 ± 0.05*	0.29 ± 0.07***	
Cl	mmol	0.29 ± 0.05	0.26 ± 0.07	0.34 ± 0.09	0.66 ± 0.15***	0.18 ± 0.07	0.18 ± 0.04	0.31 ± 0.07***	

Interval 1 : Urine collected immediately after dosing until approximately 17.00 hours.

Interval 2 : Urine collected overnight from approximately 17.00 hours to approximately 09.00 hours.

Each value represents the Mean ± SD.

Significant when compared with control: *p<0.05; **p<0.01; *** p<0.001.

lined by hypertrophic and hyperplastic epithelium (Photo. 2).

Other changes included a benign tumor of the adrenal medulla which occurred in a male in the high dosage group. This is a common age-related spontaneous tumor in this strain of rat, and the single occurrence in this study is not considered to be related to treatment. In addition, a reduced incidence of focal inflammation associated with hepatocyte degeneration in the liver was seen in males from the high dosage group. This lesion is a common background finding in male rats of this age and strain; the reduced incidence in the treated males is not considered to be of toxicological significance. There were no pathological changes in the stomach considered to be related to treatment.

Pathological changes for animals killed after 13 weeks of reversibility included pulmonary lesions characterized by bronchiolar hyperplasia, focal chronic interstitial pneumonitis and alveolar fibrosis observed in all the males and the majority of females previously treated with trientine-2HCl at 600 mg/kg/day. There was no evidence of recovery.

DISCUSSION

The toxicity of trientine-2HCl was evaluated in oral 4- or 8-week and 26-week studies in F-344 rats. There were two deaths of males receiving 1200 mg/kg/day in the 4- or 8-week study and four deaths of treated males, comprising three males receiving 600 mg/kg/day and one male receiving 175 mg/kg/day in the 26-week study. The cause of death of these animals with shallow and irregular respiration was probably due to the treatment-related changes (broncho-alveolar pneumonia and bronchiolar epithelium hypertrophy) seen in the lungs. Similar pathological changes in the lungs were also recorded for animals killed after 4 or 8 weeks of treatment. In the 26-week study, focal chronic interstitial pneumonitis, alveolar fibrosis, bronchiolar epithelium hypertrophy and bronchiolar hyperplasia of the lungs were observed at dosages of 175 mg/kg/day and 600 mg/kg/day for females and in all treated male groups killed at the end of the treatment period. The lesions recorded in the lungs of treated animals in both studies were clearly treatment-related and remained after the 13-week reversibility period. They were considered to be irreversible.

Greenman *et al.* (1996) reported chronic inflamma-

Table 9-2. Urinalysis for rats at the end of reversibility period in 26-week toxicity study of trientine-2HCl.

Sex:		Males		Females	
Dosage (mg/kg/day):		0	600	0	600
[Interval 1]					
(Number of animals)		(7)	(7)	(8)	(6)#
Volume	mL	2.3 ± 1.0	2.2 ± 0.9	2.0 ± 0.9	2.0 ± 0.5
pH		7.2 ± 0.4	7.8 ± 0.4 *	7.2 ± 0.4	7.5 ± 0.5
Na	mmol	0.14 ± 0.08	0.16 ± 0.07	0.15 ± 0.06	0.21 ± 0.11
K	mmol	0.27 ± 0.14	0.30 ± 0.08	0.26 ± 0.11	0.39 ± 0.23
Cl	mmol	0.16 ± 0.11	0.19 ± 0.07	0.17 ± 0.10	0.31 ± 0.20
[Interval 2]					
(Number of animals)		(7)	(7)	(8)	(8)
Volume	mL	3.2 ± 1.1	3.6 ± 0.9	3.5 ± 1.8	2.8 ± 1.8
pH		7.1 ± 0.7	6.8 ± 0.5	6.6 ± 0.3	6.5 ± 0.4
SG	× 10 ⁻³	1035 ± 4	1034 ± 4	1030 ± 6	1039 ± 19
Na	mmol	0.11 ± 0.07	0.10 ± 0.02	0.09 ± 0.03	0.10 ± 0.05
K	mmol	0.46 ± 0.20	0.48 ± 0.05	0.37 ± 0.13	0.33 ± 0.14
Cl	mmol	0.15 ± 0.06	0.15 ± 0.03	0.15 ± 0.05	0.17 ± 0.07

Interval 1 : Urine collected immediately after dosing until approximately 17.00 hours.

Interval 2 : Urine collected overnight from approximately 17.00 hours to approximately 09.00 hours.

Each value represents the Mean ± SD.

Significant when compared with control : *p<0.05.

: Two urine samples were insufficient to measure.

Table 10-1. Metal concentrations in rats at the end of treatment period in 26-week toxicity study of trientine-2HCl.

Sex:	Dosage (mg/kg/day):	Males				Females			
		0	50	175	600	0	50	175	600
[Plasma]									
Copper	ppm	1.64 ± 0.12	1.51 ± 0.15	1.46 ± 0.10	0.76 ± 0.11***	2.03 ± 0.13	2.10 ± 0.12	3.65 ± 3.08	2.03 ± 0.51
Iron	ppm	0.87 ± 0.37	0.32 ± 0.06*	0.52 ± 0.29	0.70 ± 0.33	1.70 ± 0.53	1.39 ± 0.36	0.20 ± 0.01***	0.26 ± 0.09***
Zinc	ppm	0.20 ± 0.12	0.15 ± 0.04	0.25 ± 0.12	0.17 ± 0.04	0.42 ± 0.08	0.39 ± 0.03	0.22 ± 0.14*	0.24 ± 0.13*
[Urine]									
Copper	ppm	0.17 ± 0.04	0.76 ± 0.43	1.05 ± 0.48**	1.16 ± 0.36**	0.39 ± 0.21	0.64 ± 0.24	2.61 ± 1.31*	5.31 ± 2.37***#
Iron	ppm	0.96 ± 0.49	0.56 ± 0.39	0.51 ± 0.59	0.38 ± 0.34	0.15 ± 0.05	0.13 ± 0.04	3.13 ± 1.58***	0.63 ± 0.46 #
Zinc	ppm	0.05 ± 0.02	0.18 ± 0.18	0.28 ± 0.22	0.84 ± 0.59**	0.44 ± 0.33	0.09 ± 0.02	0.11 ± 0.05	4.08 ± 2.74***#
[Liver]									
Copper	ppm	4.1 ± 0.2	3.7 ± 0.4	3.6 ± 0.2**	3.2 ± 0.4***	4.6 ± 0.4	4.5 ± 0.4	4.1 ± 0.3	4.0 ± 0.2 *
Iron	ppm	147 ± 15	163 ± 27	177 ± 18	156 ± 20	353 ± 34	385 ± 63	365 ± 8	494 ± 41***
Zinc	ppm	26.4 ± 2.4	27.6 ± 3.2	29.0 ± 1.6	27.6 ± 2.6	27.4 ± 1.8	28.8 ± 1.1	27.0 ± 1.9	28.6 ± 0.9
[Kidney]									
Copper	ppm	6.7 ± 0.6	6.5 ± 1.3	5.4 ± 0.4	5.2 ± 1.4	13.5 ± 6.1	10.8 ± 0.9	8.0 ± 2.3	8.2 ± 1.9
Iron	ppm	104 ± 15	119 ± 27	117 ± 7.3	157 ± 87	207 ± 39	205 ± 16	218 ± 50	379 ± 301
Zinc	ppm	20.6 ± 0.9	21.8 ± 4.0	20.0 ± 0.7	55.6 ± 76	23.6 ± 7.0	25.6 ± 2.5	25.8 ± 5.8	29.8 ± 5.8

Each value represents the Mean ±SD.

Significant when compared with control: *p<0.05; ** p<0.01; ***p<0.001.

All group means derived from five sample values, except # where four values only were available.

Repeated dose toxicity studies of trientine-2HCl in F-344 rats.

tion of the lung interstitium with no significant effect on copper levels in the plasma in B6C3F₁ mice receiving 3000 ppm trientine-2HCl in the drinking water for 90 days and fed a purified diet containing nutritionally adequate levels of copper. The lesion was not reported in mice offered a normal cereal-based diet nor in F-344 rats whether offered a normal diet or a purified diet. In F-344 rats which received a copper-deficient diet and whose copper concentrations of plasma and liver were markedly depleted, copper deficiency was found in the heart, spleen and pancreas, but no lesions were found in the lungs. Consequently, Greenman *et al.* concluded that the lung lesion was not related to copper deficiency.

In the present 26-week study, low plasma copper concentrations were seen in male rats receiving 600 mg/kg/day, with low liver copper concentrations found in animals receiving 600 or 175 mg/kg/day and high urinary copper concentration in all treated groups. This is probably associated with the pharmacological action of trientine-2HCl. The low liver copper concentrations were, however, slight and the low plasma copper concentrations were generally confined to males receiving 600 mg/kg/day. It clearly was not a response to copper

depletion since it was not similar to the lung pathology noted in copper-deficient (O'dell *et al.*, 1978) or penicillamine-treated rats (Kilburn and Hess, 1982). It is therefore unlikely that the lung lesions observed in the present studies were associated solely with low tissue copper concentrations, but that they were also associated with other actions of trientine-2HCl.

The histopathological lung changes seen in the decedent animals provide an indication of the pathogenesis of the lesions seen. Severe necrosis and regenerative hyperplasia of the epithelium lining the terminal bronchioles and acute alveolar inflammation in these animals suggest that these are early changes and that the bronchiolar epithelium and the alveolar epithelium probably represent the target cells. In the animals killed at the end of the 26-week treatment period, the acute interstitial pneumonitis which was seen in the early decedents had developed into a chronic lesion, where the alveolar walls were collapsed and replaced by fibrosis. This is indicative of a persistent inflammatory reaction or a persistent toxic effect on the alveolar pneumocytes, preventing normal repair of the alveolar lining. In a preliminary short-term study using electron microscopy, it was suggested that the primary target

Table 10-2. Metal concentrations in rats at the end of reversibility period in 26-week toxicity study of trientine-2HCl.

Sex		Males		Females	
Dosage (mg/kg/day):		0	600	0	600
[Plasma]					
Copper	ppm	1.97 ± 0.12	2.00 ± 0.30	1.28 ± 0.16	1.55 ± 0.77
Iron	ppm	0.18 ± 0.05	0.14 ± 0.02	0.37 ± 0.42	0.71 ± 1.11
Zinc	ppm	0.18 ± 0.04	0.61 ± 0.66	0.29 ± 0.17	0.22 ± 0.13
[Urine]					
Copper	ppm	0.57 ± 0.13	0.57 ± 0.37	0.28 ± 0.11	0.26 ± 0.05 #
Iron	ppm	0.55 ± 0.27	0.35 ± 0.13	2.98 ± 1.58	3.77 ± 1.56 #
Zinc	ppm	0.09 ± 0.11	0.06 ± 0.04 #	0.18 ± 0.06	0.16 ± 0.04 #
[Liver]					
Copper	ppm	3.4 ± 0.3	3.9 ± 0.4	5.4 ± 0.5	5.2 ± 0.3
Iron	ppm	151 ± 7	175 ± 18*	377 ± 45	376 ± 43
Zinc	ppm	22.4 ± 1.3	26.0 ± 1.4 **	35.0 ± 16.3	27.6 ± 0.9
[Kidney]					
Copper	ppm	8.4 ± 3.8	7.3 ± 1.2	10.5 ± 1.7	10.9 ± 1.2
Iron	ppm	138 ± 14	154 ± 28	237 ± 17	215 ± 20
Zinc	ppm	19.8 ± 2.4	21.0 ± 3.0	25.0 ± 1.6	24.0 ± 0.7

Each value represents the Mean ± SD.

Significant when compared with control: *p<0.05; ** p<0.01.

All group means derived from five sample values, except # where four values only were available.

Table 11-1. Summary of organ weights in rats at the end of treatment period in 26-week toxicity study of trientine-2HCl.

Dosage (mg/kg/day): Number of animals:	Male				Female			
	0 12	50 12	175 11	600 10	0 12	50 12	175 12	600 12
Body weight g	359.5 ± 21.7	360.2 ± 23.4	356.3 ± 17.5	321.0 ± 26.0**	183.3 ± 11.3	186.0 ± 9.2	182.0 ± 9.0	179.6 ± 11.0
Kidneys g	1.96 ± 0.15 ^{a)}	1.92 ± 0.18	1.93 ± 0.12	1.88 ± 0.19	1.14 ± 0.08	1.15 ± 0.09	1.13 ± 0.07	1.16 ± 0.08
g/100gBW	0.545 ± 0.023 ^{b)}	0.533 ± 0.022	0.543 ± 0.021	0.586 ± 0.017**	0.625 ± 0.043	0.615 ± 0.023	0.621 ± 0.021	0.648 ± 0.023
Lungs g	1.43 ± 0.20	1.62 ± 0.53	1.46 ± 0.14	1.55 ± 0.40	0.93 ± 0.19	0.88 ± 0.07	0.92 ± 0.08	0.95 ± 0.06
g/100gBW	0.399 ± 0.053	0.446 ± 0.122	0.411 ± 0.047	0.492 ± 0.166	0.509 ± 0.117	0.471 ± 0.033	0.504 ± 0.038	0.531 ± 0.041
Spleen mg	799 ± 51	770 ± 61	798 ± 43	666 ± 63**	420 ± 31	412 ± 45	426 ± 35	428 ± 31
mg/100gBW	222.2 ± 8.4	213.9 ± 14.3	224.3 ± 11.0	207.6 ± 11.1*	229.7 ± 15.5	221.1 ± 17.6	233.9 ± 13.6	238.8 ± 15.6
Thymus mg	269 ± 64	294 ± 61	281 ± 54	228 ± 56	167 ± 41	181 ± 37	204 ± 30*	184 ± 29
mg/100gBW	74.7 ± 16.7	81.9 ± 18.0	79.3 ± 16.8	71.1 ± 17.1	90.4 ± 19.4	97.4 ± 18.8	112.1 ± 15.8*	102.6 ± 14.6

Each value represents the Mean ± SD.

a) : Upper column indicates absolute organ weight.

b) : Lower column indicates organ weight relative to body weight.

Significant when compared with control : * p<0.05; ** p<0.01.

Table 11-2. Summary of organ weights in rats at the end of reversibility period in 26-week toxicity study of trientine-2HCl.

Dosage (mg/kg/day): Number of animals:	Male		Female	
	0 7	600 7	0 8	600 8
Body weight g	383.3 ± 18.7	354.2 ± 31.3	194.0 ± 11.8	185.9 ± 8.5
Kidneys g	2.11 ± 0.11 ^{a)}	2.05 ± 0.12	1.21 ± 0.08	1.23 ± 0.08
g/100gBW	0.551 ± 0.014 ^{b)}	0.582 ± 0.027*	0.623 ± 0.015	0.663 ± 0.024**
Lungs g	1.46 ± 0.15	1.49 ± 0.13	0.95 ± 0.07	1.01 ± 0.19
g/100gBW	0.381 ± 0.040	0.424 ± 0.045	0.488 ± 0.016	0.545 ± 0.106
Spleen mg	822 ± 45	793 ± 103	425 ± 25	446 ± 39
mg/100gBW	214.6 ± 9.6	224.1 ± 24.9	219.0 ± 7.8	240.0 ± 18.9*
Thymus mg	229 ± 88	271 ± 56	137 ± 34	159 ± 34
mg/100gBW	59.9 ± 22.8	76.0 ± 10.4	70.6 ± 18.3	85.5 ± 18.3

Each value represents the Mean ± SD.

a): Upper column indicates absolute organ weight.

b): Lower column indicates organ weight relative to body weight.

Significant when compared with control : * p<0.05; ** p<0.01.

Repeated dose toxicity studies of trientine-2HCl in F-344 rats.

Table 12-1. Summary of histopathological findings for rats at the end of treatment period in 26- week toxicity study of trientine-2HCl.

Sex:	Males				Females			
	0	50	175	600	0	50	175	600
Dosage (mg/kg/day):	12	12	12(1)	12(2)	12	12	12	12
Number of animals:								
Adrenals Med L&R	12	0	0	12(2)	12	0	0	12
B-Phaeochromocytoma	0			1	0			0
Left kidney	12	0	0	12(2)	12	0	0	12
Cortico-medullary mineralization	1			0	4			3
Basophilic cortical tubules	1			0	0			0
Liver x 2	12	0	0	12(2)	12	0	0	12
Focal inflammation with associated hepatocytic degeneration	10			1***	1			0
Focal necrosis with inflammatory infiltrate	1			0	0			0
Chronic inflammatory cells within the portal area	0			1	0			0
Lungs x 2	12	12	12(1)	12(2)	12	12	12	12
Perivascular oedema	0	0	(1)	3(2)	0	0	0	0
Necrosis/regenerative hyperplasia of terminal bronchioles	0	0	3(1)	3(2)	0	0	0	0
Acute interstitial pneumonitis	0	0	2(1)	3(2)	0	0	0	0
Focal chronic interstitial pneumonitis	1	7*	9**	10***	0	0	6*	12***
Alveolar fibrosis	0	4	8**	10***	0	0	4	11***
Alveolar hemorrhage	0	0	(1)	5(2)*	0	0	0	0
Bronchiolar hyperplasia / epithelialization of alveolar wall	0	2	10(1)***	11(1)***	0	0	3	9***
Bronchiolar epithelium hypertrophy	0	0	7(1)**	10(2)***	0	0	0	3
Focal pneumonia	1	0	0	0	0	0	0	0
Granuloma(s)	2	0	0	0	0	0	0	0
Stomach x 2	12	12	12(1)	12(2)	12	12	12	12
Keratinized region: Acute inflammation	0	0	0	0	0	0	0	1

Significant when compared with control : * p<0.05; ** p<0.01; *** p<0.001.

The figures in parentheses show the number of rats found dead or killed in *extremis*.

cells might be Type I and Type II alveolar cells (unpublished data). Foster *et al.* (1990) reported that exposure of rat lungs to spermidine, one of the endogenous polyamines, resulted in a specific necrosis of Clara cells of the bronchiolar epithelium and alveolar Type II cells and then progressed through alveolitis to a fully developed subchronic pneumonia. The endogenous polyamines have been shown to be accumulated into Clara cells of the bronchiolar epithelium and alveolar epithelial Type I and Type II cells via the endogenous polyamine transport system (Wyatt *et al.*, 1988). In the distribution study of trientine-2HCl by a single oral administration with ^{14}C -trientine-2HCl, specific accumulation of radioactivity in the lung was not demonstrated (Takeda *et al.*, 1995). Nevertheless, trientine-2HCl, a kind of polyamine, could be taken into bronchiolar epithelium and/or alveolar epithelium by an endogenous polyamine uptake process and be accumulated in the specific tissue site of the lung. Polyamines are generally irritating agents for mucous membranes, upper respiratory tract and skin (Lenga, 1988; Maissey *et al.*, 1988). In the 4- or 8-week study, the histopathological changes observed in the stomach (submucosal inflammation within the glandular region) indicate that trientine-2HCl was slightly irritant. Therefore, it is likely that chronic interstitial pneumonitis is caused by the cytotoxic effect of trientine-2HCl which was accumulated into the bronchiolar epithelial cells and the alveolar pneumocytes.

The changes seen in urinary electrolyte outputs in

both studies are possibly due to the hydrochloride nature of trientine-2HCl. Another study compared the dihydrochloride and tetrahydrochloride forms of trientine. Chloride output in the urine of animals receiving the tetrahydrochloride form was almost twice as much as that of animals receiving the dihydrochloride (unpublished data). The increase in sodium and potassium output is probably secondary to the increase in chloride output. The high acidity of the urine is probably associated with the increased electrolyte output. The low alkaline phosphatase activity, a zinc enzyme, observed at the middle and high dosages in both studies, may be related to the pharmacological activity of trientine-2HCl. This is a known effect of metal chelators (Khandelwal *et al.*, 1987). In the 4- or 8-week study, high alanine amino-transferase activity and high triglyceride and cholesterol concentrations were seen in rats receiving 1200 mg/kg/day, but there was no evidence of these findings in the 26-week study at dosages up to 600 mg/kg/day. In both the 4- or 8-week and 26-week studies, increased packed cell volumes, hemoglobin concentrations and erythrocyte counts were observed in high dosage animals; however, these increases were minor and are probably of little toxicological significance. Slightly increased relative kidney weights for high dosage animals were observed in both studies. This change was still evident at the end of the reversibility period. Since there was no evidence of histopathological findings in both studies, the increased kidney weights probably reflect some degree of hyper-

Table 12-2. Summary of histopathological findings for rats at the end of reversibility period in 26-week toxicity study of trientine-2HCl.

Sex:	Males		Females	
Dosage (mg/kg/day):	0	600	0	600
Number of animals:	8(1)	8(1)	8	8
Lungs x 2				
Perivascular edema	0	(1)	0	0
Necrosis/regenerative hyperplasia of terminal bronchioles	0	(1)	0	0
Acute interstitial pneumonitis	0	(1)	0	0
Focal chronic interstitial pneumonitis	0	7**	1	7*
Alveolar fibrosis	0	7**	0	7**
Alveolar hemorrhage	2	2(1)	0	0
Bronchiolar hyperplasia/epithelialization of alveolar wall	0	8(1)***	0	7**
Bronchiolar epithelium hypertrophy	0	7**	0	2
Granuloma(s)	4	0	2	0
Monocytic leukemia	(1)	0	0	0

Significant when compared with control : * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$.

The figures in parentheses show the number of rats found dead or killed *in extremis*.

Repeated dose toxicity studies of trientine-2HCl in F-344 rats.

Photo 1. Lung from a male rat treated orally with triene-2HCl 600 mg/kg/day for 26 weeks: Focal chronic interstitial pneumonitis. H.E. staining, $\times 17$.

Photo 2. Lung from a male rat treated orally with triene-2HCl 600 mg/kg/day for 26 weeks: Bronchiolar hyperplasia / epithelialization of alveolar wall and bronchiolar epithelium hypertrophy. H.E. staining, $\times 17$.

trophy with the essentially pharmacological changes indicative of the high electrolyte output.

In the 4- or 8- week study, incidence of the histopathological changes in the stomach was decreased for animals receiving 100 and 350 mg/kg/day that were killed after 8 weeks of treatment when compared with those killed after 4 weeks, and no changes were observed in the stomach in the 26-week study, indicating the development of some degree of tolerance. These changes are considered to be of no toxicological significance.

It is concluded that oral administration of trientine-2HCl to rats was associated with death and irreversible toxic changes in the lung. A dosage of 50 mg/kg/day was considered to be the non- observed adverse effect level (NOAEL) for females and for males the NOAEL was considered to be less than 50 mg/kg/day.

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