5.4 Repeated Dose Toxicity

Species: rat **Sex:** male/female
Strain: other: Harlan-Wistar
Route of administration: oral feed
Exposure period: 7 days
Frequency of treatment: daily ad libitum
Post exposure period: no data
Doses: m: 0.5, 1.23, 2.98 g/kg b.w.; f: 0.47, 1.38, 2.63 g/kg b.w.
Control Group: no data specified
NOAEL: ,5

Method: other: 5 rats per dose and sex
GLP: no data

Test substance: no data

Remark: LOEL: 1.23 (m) and 1.38 (f) mg/kg b.w./day
remarks: no deaths occurred
Result: highest dose:
depression of body weight gain, decrease of relative and absolute liver weights, increase of relative kidney weights.
medium dose:
increase of relative kidney weights.

17-OCT-1994 (28)

Species: rat **Sex:** male/female
Strain: Fischer 344
Route of administration: drinking water
Exposure period: 90 d
Frequency of treatment: daily
Post exposure period: no
Doses: 0, 120, 600, 3000 ppm (see remarks)
Control Group: other: concurrent no treatment (diet: cereal based NIH-31, purified AIN-76A, Cu-deficient AIN-76A)
NOAEL: = 3000 ppm

Method: other: 18 rats/sex and dose group, different diets: cereal based (NIH-31) or purified (AIN-76A) diet; hematology and plasma chemistry; necropsy and histopathology; statistical analyses
Year: 1996
GLP: no data
Test substance: other TS: trientine-2HCl: purity: > 99 %

Remark: test substance consumption:
NIH-31 diet: f:14, 70, 352 mg/kg bw; m:10, 55, 276 mg/kg bw
AIN-76A diet: f:13, 60, 323 mg/kg bw; m:10, 53, 270 mg/kg bw
Result: no death occurred; pobabely attributed to dosing with trien-2HCL: females: a significant trend toward an increased prevalence of uterine dilatation; no other findings

23-JUN-1997 (53)

Species: rat **Sex:** female
Strain: Wistar
Route of administration: dermal
Exposure period: 17 days

Frequency of treatment: once daily (3rd - 19th day of gestation)
Post exposure period: no
Doses: ca. 4 mg/rat and day
Control Group: yes

Method: other: 10 rats per group. One drop of the test substance was rubbed into the shaved skin

GLP: no data
Test substance: no data

Remark: LOEL: no data
Result: pregnant and nonpregnant rats: reduced weight gain, progressive emaciation, apathy, lack of appetite, local inflammatory symptoms such as erythema, edema and superficial erosions. pregnant rats: increase of plasma sialic acid; increased activity of lactate dehydrogenase, aspertate aminotransferase and acid phosphatase in the serum; decreased plasma activity of alkaline phosphatase; reduced haptaglobin concentration; increased acti- vity of leucylnaphthylamidase in amniotic fluid. nonpregnant rats: decreased total plasma protein and elevated concentrations of seromucoid a. haptaglobin; in the serum increa- sed activity of lactate dehydrogenase, leucylnaphthylamidase and alkaline phosphatase; inhibited activity of aspartate and alanine aminotransferase.

(54)

Species: rat **Sex:** female
Strain: Wistar
Route of administration: dermal
Exposure period: 17 days
Frequency of treatment: once daily
Post exposure period: no
Doses: ca 4 mg/rat and day
Control Group: yes

Method: other: 10 rats per group. No data about stage of pregnancy in pregnant rats. One drop of test substance was rubbed into the shaved skin.
GLP: no data
Test substance: no data

Remark: LOEL: no data
Result: pregnant and nonpregnant rats:
weight loss, hyperemia of liver and kidneys, dermis and subcutaneous tissue with inflammatory infiltrates.
pregnant rats: aspartate aminotransferase activity in the liver inhibited.
nonpregnant rats: increased activity of gammaglutamyltranspeptidase in the kidney and aspartate and alanine aminotransferases in the liver.

(55)

Species: rat **Sex:** no data
Strain: no data
Route of administration: oral unspecified
Exposure period: a) 4 months b) 10 months
Frequency of treatment: a) no data b) daily
Post exposure period: no data

Doses: a) 215 or 430 mg/kg b) 0.8 or 4 mg/kg
Control Group: no data specified

Method: other: no data
GLP: no data
Test substance: no data

Remark: LOEL: a) 215 mg/kg b.w. b) 0.8 mg/kg b.w./day, 10 months no dose effect relation; abstract, no further information available.
Result: 4 months both doses:
Excitability of the central nervous system decreased. Plasma levels of hippuric acid, protein and hemaglobin were decreased. Inhibited activities of catalase and peroxidase.
10 months both doses:
Increased excitability, stimulated tactile reflexes. Antitoxic, carbohydrate and protein function of the liver disturbed. Transient inhibition of nicotinamide coenzymes and stimulation of cytochrome oxidase.

17-OCT-1994 (31)

Species: mouse **Sex:** male/female
Strain: B6C3F1
Route of administration: drinking water
Exposure period: 90 d
Frequency of treatment: daily
Post exposure period: no
Doses: 0, 120, 600, 3000 ppm (see remarks)
Control Group: other: concurrent no treatment, (diet: cereal based NIH-31, purified AIN-76 A, Cu-deficient AIN-76A)
NOAEL: = 600 ppm

Method: other: 20 mice/sex and dose group; different diets: cereal based (NIH-31) or purified (AIN-76A); hematology and plasma chemistry; necropsy, histopathology, statistical analyses
Year: 1996
GLP: no data
Test substance: other TS: trientine-2HCl; purity: > 99 %

Remark: test substance consumption:
NIH-31 diet: f:22,107, 551 mg/kg bw; m:22,107, 487 mg/kg bw
AIN-76A diet: f:19, 99, 483 mg/kg bw; m:17, 92, 443 mg/kg bw
Result: diet AIN-76A, 3000 ppm: chronic interstititial inflammation and alveolar histocytic infiltration of the lung, spleen hemapoetic cell proliferation, liver periportal fatty change, kidney weight reduction, reduced renal cytoplasmatic vacuolization, body weight gain reduction

27-JAN-1998 (53)

Species: guinea pig **Sex:** female
Strain: no data
Route of administration: dermal
Exposure period: 55 days
Frequency of treatment: once daily
Post exposure period: no
Doses: ca.4 mg/animal and day
Control Group: yes

Method: other: starting exposition in pregnant guinea pigs on day 10 of gestation. One drop of the test substance was rubbed into the shaved skin.
GLP: no data
Test substance: no data

Remark: LOEL: no data
remarks: 6 out of 10 nonpregnant and 2 out of 9 pregnant exposed guinea pigs died before end of experiment. No further information about toxic effects available.

Result: pregnant guinea pigs:
activity of gammaglutamyltranspeptidase significantly elevated in kidney and blood.
nonpregnant guinea pigs:
significantly increased activity of liver aspartate aminotransferase.

(56)

Species: guinea pig **Sex:** female
Strain: no data
Route of administration: dermal
Exposure period: once daily for 10 days, then every second day for 45 days
Post exposure period: no
Doses: ca.4 mg/animal and day
Control Group: yes

Method: other: 11 animals/group; exposure started on day 10 of gestation; one drop of the test substance was rubbed into the shaved skin
GLP: no data
Test substance: no data

Remark: LOEL: no data
Result: 7 out of 11 pregnant and 7 out of 11 nonpregnant guinea pigs died within the first 10 days. Surviving pregnant and nonpregnant animals showed weight loss with advanced emaciation; skin revealed inflammatory alterations indicated by erythema, edema and erosion. Surviving and nonsurviving ainmals showed all fatty degeneration of the liver, congestion of the kidney and brain, and brain edema. Pregnant animals showed necrotic changes in the placenta and miscarriage or mortification of fetuses.

(57)

Species: other: see remarks **Sex:** no data
Strain: no data
Route of administration: inhalation
Exposure period: 1 h/d for 2 weeks, 5 d a week
Post exposure period: no data
Doses: 0.4 ml in 5 ml ethanol as aerosol in a 400 l chamber
Control Group: no data specified

Method: other: 1 guinea pig, 1 rabbit, 2 rats, 4 mice were exposed together in one chamber.
GLP: no data
Test substance: no data

Remark: LOEL: no data
no further information available
Result: no effects
17-OCT-1994 (29)

5.5 Genetic Toxicity 'in Vitro'

Type: Ames test
System of testing: Salmonella typhimurium, TA 100, TA 1535
Metabolic activation: with and without
Result: positive

Method: other: no data
GLP: no data
Test substance: no data

Remark: abstract, no further information available
(58)

Type: Ames test
System of testing: Salmonella typhimurium, TA 100,
Metabolic activation: no data
Result: positive

Method: other: no data
GLP: no data
Test substance: no data

Remark: 0.07 revertants per nmole;
abstract, no further information available
(59)

Type: Bacterial gene mutation assay
System of testing: Escherichia coli
Metabolic activation: without
Result: positive

Method: other: no data
GLP: no data
Test substance: no data
(60)

Type: Ames test
System of testing: Salmonella typhimurium, TA 92, 98, 100
Metabolic activation: without
Result: positive

Method: other: no data
GLP: no data
Test substance: no data
(60)

Type: Ames test
System of testing: Salmonella typhimurium, TA 98, 100, 1535, 1537, 1538
Metabolic activation: with and without
Result: positive

Method: other: no data
GLP: no data

Test substance: other TS: purified TETA-2Hydrochloride

(61)

Type: Ames test
System of testing: Salmonella typhimurium, TA 98, 100, 1535, 1537
Metabolic activation: with and without
Result: positive

Method: other: preincubation assay
GLP: no data
Test substance: other TS: technical grade (68.1%)

(62)

Type: Ames test
System of testing: Salmonella typhimurium, TA 98, 100, 1535, 1537, 1538
Metabolic activation: with and without
Result: positive

Method: other: no data
GLP: yes
Test substance: other TS: techn. grade; 2 samples: 56.4 and 68.5% purity

(63) (64)

Type: Mammalian cell gene mutation assay
System of testing: CHO cells
Metabolic activation: with and without
Result: positive

Method: other: no data
GLP: no data
Test substance: other TS: purity 79.15%

Remark: no clear dose-response relationship

(65)

Type: Mammalian cell gene mutation assay
System of testing: CHO cells
Metabolic activation: with and without
Result: negative

Method: other: no data
GLP: no data
Test substance: other TS: purity 99.42%

(66)

Type: Sister chromatid exchange assay
System of testing: CHO cells
Metabolic activation: with and without
Result: positive

Method: other: no data
GLP: no data
Test substance: other TS: purity 99.42%

(66)

Type: Unscheduled DNA synthesis
System of testing: rat hepatocytes
Metabolic activation: without
Result: positive

Method: other: no data
GLP: no data
Test substance: other TS: purity 99.42%

(66)

Type: Sister chromatid exchange assay
System of testing: CHO cells
Metabolic activation: with and without
Result: positive

Method: other: no data
GLP: no data
Test substance: other TS: purity 79.15%

(65)

Type: Unscheduled DNA synthesis
System of testing: rat hepatocytes
Metabolic activation: without
Result: positive

Method: other: no data
GLP: no data
Test substance: other TS: purity 79.15%

(65)

Type: Sister chromatid exchange assay
System of testing: CHO cells
Metabolic activation: with and without
Result: positive

Method: other: no data
GLP: no data
Test substance: other TS: purity 56.4%, technical grade

Remark: with metab. activation only at the lowest concentration (0.5 g/l) significant increase of SCEs/chromosome; no increase at 0.6 and 0.8 g/l.

(67)

5.6 Genetic Toxicity 'in Vivo'

Type: Drosophila SLRL test
Species: Drosophila melanogaster **Sex:** no data
Route of admin.: unspecified
Exposure period: no data
Doses: no data

Method: other: no data
GLP: no data
Test substance: no data

Result: no effects

(68)

Type: Micronucleus assay
Species: mouse **Sex:** male/female
Route of admin.: i.p.
Exposure period: single injection
Doses: 185, 370, 600 mg/kg

Method: other: Bushy Run Research Center standard protocol
GLP: yes
Test substance: other TS: purity 68.5%, technical grade

Result: not clastogenic

(69)

Type: Micronucleus assay
Species: mouse **Sex:** no data
Route of admin.: i.p.
Exposure period: single injection
Doses: 130, 190, 250 mg/kg

Method: other: according to Schmid, W., Mitt. III der Komm. fuer Mutagenitaetsfragen, 53 (1975)
GLP: no data
Test substance: other TS: purified TETA-Dihydrochloride

Result: not clastogenic

(61)

Type: Micronucleus assay
Species: mouse **Sex:** no data
Route of admin.: oral unspecified
Exposure period: single application
Doses: 1500, 3000, 6000 mg/kg

Method: other: according to several published methods
GLP: no data
Test substance: other TS: purified TETA-2Hydrochloride

Result: not clastogenic

(61)

5.7 Carcinogenicity

Species: mouse **Sex:** male
Strain: other: C3H/HeJ
Route of administration: dermal
Exposure period: life-time
Frequency of treatment: 3 times a week
Post exposure period: no
Doses: ca. 1.2 mg/mouse and application
Control Group: other: deionized water

Method: other: see remarks
GLP: no data
Test substance: other TS: purity 79.15% (analytic)

Remark: method: no further data available
remarks: 50 animals per group; 0.025 ml of 5% aqueous solution applied; dose highest one that resulted in neither skin irratation nor reduced weight gain. No increased mortality. Dosage very low compared to LD50.
Result: No treatment related skin tumors, no evidence of increased incidence of any other tumor.

(70)

Species: mouse **Sex:** male
Strain: other: C3H/HeJ
Route of administration: dermal
Exposure period: 2 years
Frequency of treatment: 3 times/week
Doses: 0, 0.2 or 2.0 % in ethanol

Remark: 50 animals/group
Result: No effects were observed on any parameter, including mortality, body weights and incidence of tumorous or non-tumorous lesions.
Source: DOW Europe S.A., Switzerland
24-MAY-1994 (71)

5.8.1 Toxicity to Fertility

5.8.2 Developmental Toxicity/Teratogenicity

Species: rat **Sex:** female
Strain: Sprague-Dawley
Route of administration: gavage
Exposure period: day 6-15 of gestation
Frequency of treatment: once daily
Doses: 75, 325, 750 mg/kg
Control Group: yes

Method: other: test substance diluted in water
GLP: no data
Test substance: other TS: purity > 98%

Remark: no further information available
Result: No substance related effects on dams or fetuses, except increased fetal body weight at 750 mg/kg (no data about significance).
(72)

Species: rat **Sex:** female
Strain: Sprague-Dawley
Route of administration: oral feed
Exposure period: day 0-21 of gestation
Frequency of treatment: daily ad libitum
Doses: 0.17, 0.83, 1.66% in the diet (170, 830, 1660 mg/kg b.w. and day)
Control Group: yes

GLP: no data
Test substance: other TS: purity > 99%, TETA-4Hydrochloride

Remark: litter size unchanged, all described effects significant and dose related. Authors comment: teratogenicity of the drug in part due to induced Cu deficiency and Zn toxicity.

Result: Controls (n=7): no resorbed or abnormal fetuses.
0.17%
dams(n=5): no effects except reduced liver copper and increased kidney zinc concentration. Fetuses: 5.8% resorbed (3/52), whole fetus and liver Zn conc. elevated, Cu liver conc. reduced.

0.83%
dams (n=9): reduced weight gain, decreased Cu conc. in liver and plasma, Zn conc. increased in kidney and muscle.
Fetuses: 8.7% resorbed (7/93), 25,6% abnormalities (22/86) like hemorrhage and edema, Cu decreased in whole body, liver and placenta, Zn concentration elevated in whole body and liver.
1.66%
dams (n=5): reduced food consumption;
highly signif. reduced weight gain and copper concentration in liver and plasma. Zn conc. in kidney and muscle, manganese conc. in muscle and iron conc. in liver increased.
Fetuses: 18.8% resorbed (9/48); 100% abnormalities (39/39) like hemorrhages, edema, reduced ossification of caudal vertebrae and phalanges; fetal weight and length reduced.
Trace elements same results as in medium dose.
(73) (74) (75) (76)

Species: rat **Sex:** female
Strain: Sprague-Dawley
Route of administration: oral feed
Exposure period: day 0-21 of gestation
Frequency of treatment: daily ad libitum
Doses: 0, 0.83 or 1.67% in diet combined with 0.05 or 0.5 mg Cu/kg diet
Control Group: yes

Method: other: 4 rats per group
GLP: no data
Test substance: other TS: purity > 99%

Remark: litter size not altered by test substance or Cu administration.
Authors comment: teratogenicity of the test substance in part due to induced Cu deficiency. Doses used here correspond to 830 or 1670 mg per kg b.w. and day.
Result: Maternal weight gain and fetal weight and length were significantly decreased at 1.67% without improvement by copper supplement. Frequency of resorption not different in any group. Significant incidence of fetal abnormalities (69%, 27 out of 39 fetuses) due to 1.67% in combination with the low Cu concentraion was lowered to 6.5% (3/46) by high Cu concentration. Types of abnormalities: hemorrhage, edema, hydronephrotic kidneys, micrognathia and domed skulls. The lowered teratogenetic effect of 1.67% was correlated with an increase in maternal and fetal tissue copper levels by Cu supplement.
Increased maternal and
fetal zinc levels due to the test substance were not altered by Cu coadministration.
(77) (78) (79)

Species: rabbit **Sex:** female
Strain: other: New Zealand
Route of administration: dermal
Exposure period: day 6-18 of gestation
Frequency of treatment: 6 h each day

Doses: 5, 50, 125 mg/kg dissolved in 2 ml distilled water

Control Group: yes
NOAEL Teratogenicity: 125 mg/kg bw

Method: other: 22 rabbits per group; application occlusive
GLP: no data
Test substance: other TS: purity 95%

Result: No embryotoxic or teratogenic drug related effects at any dose.
Maternal toxicity:
125 mg/kg induced delayed weight gain and death of 2 out of 22 rabbits. Strong local irritations of the skin at 50 and 125 mg/kg and slight reversible irratations at 5 mg/kg. No reduction of copper concentrations in urine and plasma.
(80)

Species: other: chicken **Sex:** no data
Strain: other: White Leghorn
Route of administration: other
Exposure period: once in 3 days old embryos
Doses: 0.051, 0.102, 0.204 or 0.408 mg per egg dissolved in 5 ul acetone
Control Group: other: solvent

Method: other: injection on the inner shell membrane
GLP: no data
Test substance: other TS: technical grade

Result:

	deaths of embryos	malformed survivors
0.051 mg	1 out of 30	2 out of 29
0.102 mg	3/30	3/27
0.204 mg	10/30	4/20
0.408 mg	20/20	----
acetone	1/100	0/100

Malformations occurred in the eyes, wings and abdominal wall. Oedema, enlarged lymph sacs and stunting and twisting of the backbone. ED50 for embryotoxicity: 0.155 mg per egg.
(81)

5.8.3 Toxicity to Reproduction, Other Studies

5.9 Specific Investigations

5.10 Exposure Experience

Remark: TETA-2Hydrochloride is used in the therapy of Wilson''s disease (inherited metabolic desease characterised by copper accumulation predominantly in liver, cornea, brain, and kidney) when the drug of choice (Penicillamine) is not tolerated. All authors reported no serious side effects.
(82) (83) (84) (85) (86) (87) (88) (89) (90) (91)

Remark: In primary biliary cirrhosis treatment TETA is an unsuitable drug due to gastrointestinal side effects, skin rash and rhabdomyolysis (one out of 4 patients 48 h after 1. dose)

(92)

Remark: There was no evidence of teratogenicity in 4 patients who became pregnant while taking TETA-2Hydrochoride against Wilson''s disease (6 pregnancies).

(89)

Remark: 6 out of 20 employees working with ethoxylin cast resin and the hardener TETA suffered from work related eczematous dematosis. 8/20 showed slight skin irratations like erythemaand itching. In epicutaneous skin test 5 out of 6 workers with strong dermatosis were sensitized to TETA (technical grade).

(93)

Remark: Serum monoamine oxidase activity in 15 workers handling with epoxy resin and hardener TETA was significantly elevated compared to a control group. Increased activity reflect possibly increased amine metabolism in the connective tissue.

(94)

Remark: 12 workers exposed to araldite and hardener TETA were examined 2 to 4 times at intervals of 6 months. After 1 year there was a decrease in the relative percentage of lymphocytes and a corresponding increase in neutrophils. 5 workers reported subjective symptomes like drowsiness, headache, gastric pain, fatigue, weakness and decreased appetite. 7 showed dermatosis.

(95)

Remark: No significant improvement occurred in hand eczema of 23 nickel-sensitive patients treated with 300 mg TETA/d in a double blind study.

(96)

Remark: Plasma levels were measured in 4 male and 4 female patients receiving treatment for excess copper. Maximal plasma levels of 0.3- 15 mg/l (male) and 1.0- 2.2 mg/l (female) were seen 3 h after oral administration of 8.3 mg/kg b.w..

The free form of the drug was not detected, indicating chelation with metal ions (predominantly copper).
test substance: TETA-2Hydrochloride

(97)

Remark: Using the oral copper loading test and the 24 h urine excretion test on patients with Wilson''s disease it could be
shown, that longterm therapy with 1.2 g/d TETA (more than 3 months) led to a decreased intestinal copper absorption and to an increased urine copper excretion.
test substance: TETA-2Hydrochloride

(98)