**Marketing Authorisation Application**

# MODULE 2.4
# NONCLINICAL OVERVIEW

## Trientine 200 mg hard capsules

## (contains 300 mg trientine dihydrochloride)

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## GLOSSARY OF ABBREVIATIONS (page 1)

| Abbreviations | Definitions | Abbreviations | Definitions |
|---|---|---|---|
| % | Percentage | HPLC | High-Performance Liquid Chromatography |
| °C | Celsius | HPRT | Hypoxanthine (Guanine) Phosphoribosyl Transferase |
| $^{14}C$ | Carbon-14 | hr | Hours |
| µg | Microgram | i.v. | Intravenous |
| ACZ | Acetazolamide | IARC | International Agency for Research on Cancer |
| AD | Alzheimer Disease | $IC_{50}$ | Inhibitory Concentration |
| AUC | Area Under Curve | ICH | International Council for Harmonisation of Technical |
| ATP | Adenosine Triphosphate | ICR | Institute of Cancer Research |
| BALB | Bagg Albino | IgA | Immunoglobulin A |
| BCH | 2-Aminobicyclo [2,2,1] Heptane-2-Carboxylic Acid | Kg | Kilogram |
| BCRP | Breast Cancer Resistance Protein | LEC | Long-Evans Cinnamon |
| Bw | Body Weight | $LD_{50}$ | Lethal Dose |
| CNS | Central Nervous System | MAPK | Mitogen-Activated Protein kinases |
| CR | Complete Response | MAT | N-Acetyltriethylenetetramine |
| Cu/Cu-II | Copper | MCF- 7 | Breast Cancer Cells |
| CuCl2 | Copper Chloride | Mg | Milligram |
| DAT | Desaminotyrosine | $mg/m^2$ | Milligram Per Square Metre |
| DNA | Deoxyribonucleic Acid | $MgC1_2$ | Magnesium Chloride |
| DN | Diabetic Nephropathy | mg/mL | Milligram Per Millilitre |
| EU | European Union | Min | Minutes |
| Fe | Ferrous | mM | Millimolar |
| FSM | Furosemide | MN | Micronucleus |
| GI | Gastrointestinal | mRNA | Messenger Ribonucleic Acid |
| GSH | Glutathione | MPC | Maximum Permissible Concentration |
| GST's | Glutathione Transferases | MTD | Maximum Tolerated Dosage |
| h | Hour | NA | Not Applicable |
| H1 | Histamine-1 | NADPH | Nicotinamide Adenine Dinucleotide |
| HAI | Hepatic Arterial Infusion | NBL | Neuroblastoma |
| HCC | Hepatocellular Carcinoma | NC | No Change |
| HCL | Hydrochloride Acid | No. | Number |

## GLOSSARY OF ABBREVIATIONS (page 2)

| Abbreviations | Definitions | Abbreviations | Definitions |
|---|---|---|---|
| NOAEL | No Observed Adverse Effect Level | Sig | Significance |
| OECD | Organisation for Economic Co-operation and Development | SmPC | Summary of Product Characteristics |
| PD | Pharmacodynamics | SSBs | Single Strand Breaks |
| PDE | Permitted Daily Exposure | TNF-α | Tumour Necrosis Factor Alpha |
| PPM | Parts per Million | $T_{1/2}$ | Half-lives |
| PSP | Phenolsulfonphthalein | TCM | Trichlormethiazide |
| pH | Potential of Hydrogen | T2DM | Type 2 Diabetes Mellitus |
| QT | Qualification Threshold | TETA | Triethylenetetramine |
| RT-PCR | Reverse Transcription Polymerase Chain Reaction | W | Week or Weeks |
| s.c. | Subcutaneously | WHOCC | World Health Organisation Collaborating Centre For Drug Statistics Methodology |
| S.E.M. | Standard Error of Measurement | WD | Wilson Disease |
| SCE | Sister Chromatid Exchange | yr | Year or Years |
| SIDS | Screening Information Dataset | Zn | Zinc |
| SD | Standard Deviation | | |

# 2.4.1 OVERVIEW OF THE NONCLINICAL TESTING STRATEGY

### 2.4.1.1 Introduction

Each 'Trientine 200 mg hard capsule' manufactured by Waymade plc, UK, contains 300 mg trientine dihydrochloride (TETA 2HCl) as the active ingredient, which is equivalent to 200 mg trientine base.

The proposed dosing regimen is 4 to 8 capsules (800 - 1600 mg), administered 2 to 4 times a day in adults and 2 to 5 capsules (400 -1000 mg), administered 2 to 4 times a day in children. The product is titrated to target according to clinical response and different copper parameters.

The indication applied for is the same as that for the reference product. 'Trientine 200 mg hard capsule' is indicated for the treatment of Wilson's disease (WD) in patients intolerant to D-penicillamine therapy, in adults, adolescents and children aged 5 years and older.'

The pharmacotherapeutic group is "various alimentary tract and metabolism products"; ATC code: A16AX12. Trientine is a chelating agent that acts due to a dual mechanism of action: primarily by promoting urinary copper excretion and to a lesser extent by reducing copper absorption from the gastrointestinal tract (and thus promoting faecal copper excretion).

Trientine was introduced in 1969 as an alternative copper chelating agent for the treatment of WD in patients who were intolerant to penicillamine. Trientine hydrochloride has been registered in the US and the United Kingdom (UK) since 1985 for the treatment of WD in patients who are intolerant of penicillamine. The sponsor's product, Trientine Hydrochloride Capsules USP 250 mg (Navinta LLC, ANDA #21125) was registered as a generic medicine to the innovator product SYPRINE (NDA #019194) in the US on 16 January 2019. Trientine was designated as an orphan drug for the treatment of WD (Submission No. PM-2019-03582-1-3) for the sponsor Waymade Australia Pty Limited on 7 August 2019.

The current marketing authorisation application is for 'Trientine 200 mg hard capsules', manufactured by Waymade Plc, UK, as a generic product, in accordance with the article 10(1) of the Directive 2001/83/EC of The European Parliament And of the Council. Each Trientine 200 mg hard capsule contains 300 mg trientine dihydrochloride, which is equivalent to 200 mg trientine base, and it is indicated for the treatment of WD in patients intolerant to D-Penicillamine therapy in adults, adolescents and children aged 5 years or older. The basis for this marketing authorisation application is the bioequivalence of this product with the reference medicinal product, 'Cufence 200 mg hard capsules' (each hard capsule of which also contains 300 mg trientine dihydrochloride, equivalent to 200 mg trientine base) manufactured by Univar BV, The Netherlands (EU/1/19/1365/001). No new toxicological, pharmacological or clinical data has therefore been presented in support of this application other than some discussions on the pharmacology, efficacy and safety of the product as derived from the public domain.

Regulatory advice was sought from MHRA on the legal basis of the application and scope of presented data to support the application [Scientific advice letter 2191, MHRA]. MHRA has agreed that submission under 10(1) article will be appropriate provided bioequivalence results were convincingly demonstrated and recommended to supplement bioequivalence results with review of the published literature on use of trientine in WD. The Applicant has followed the advice and presented in current dossier results of bioequivalence study and comprehensive literature review on pharmacology, efficacy and safety of trientine.

### 2.4.1.2 Wilson's disease (WD) and its management

WD is an inherited autosomal recessive disorder of copper balance leading to hepatic damage and neurological disturbance of variable degree [Huster D, 2010]. The World Health Organization (WHO) estimates that the global prevalence of WD is 1/10,000 to 1/30,000. Some parts of Europe such as Romania and Sardinia report highest prevalence (370–885 per million) [Nagral A et al, 2019].

WD presents symptomatically at any age, with the majority between 5 and 35 years and asymptomatic patients are most often detected by family screening [EASL, 2012]. Symptoms at the time of the initial presentation, and those that subsequently develop are most commonly categorised as hepatic or neurologic/neuropsychiatric [Cufence, EPAR].

Untreated, Wilson disease is universally fatal. Copper accumulation in the liver eventually leads to the development of cirrhosis. Among patients with neurologic Wilson disease, neurologic disease may progress until the patient becomes severely dystonic, akinetic and mute. Progression is usually gradual, but sudden deterioration may also occur. The majority of patients will die from liver disease (cirrhosis or acute liver failure), while the remainder die due to complications due to progressive neurologic disease [Cufence, EPAR].

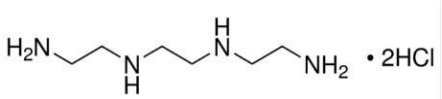
D-penicillamine (DPA) was the first drug used as a copper chelating agent [Taylor RM et al, 2009]. DPA has been a standard therapeutic drug for WD. However, it has several adverse effects which may appear in 10-30% of DPA-treated patients, and 10% of the patients with WD cannot tolerate long-term DPA therapy. The trientine salt TETA 2HCl was introduced in 1969 as a copper chelator alternative to D-penicillamine [Walshe JM,1982]. Subsequent clinical trials have indicated that trientine is effective for WD and it is less toxic than DPA [Saito H et al, 1991]. Trientine was also effective in maintaining stable hepatic and neurologic disease condition in patients with WD. Trientine hydrochloride is a copper chelating agent that aids in the elimination of copper from the body by forming a stable complex that is readily excreted by the kidneys. Trientine hydrochloride may also chelate copper in the intestinal tract and thus, inhibit copper absorption [Weber-Schondorfer C, 2015]. Trientine is generally safe and well tolerated [Weiss KH et al, 2019].

# 2.4.2 PHARMACOLOGY

## 2.4.2.1 Chemical structure and physiochemical characteristics of trientine dihydrochloride

Trientine hydrochloride is a chelating compound for removal of excess copper from the body. It is a white to pale yellow crystalline hygroscopic powder. The ATC code is: A16AX12. The chemical structure and physiochemical characteristics of trientine dihydrochloride are shown in Table 1.

**Table 1. Structure and physiochemical characteristics of trientine dihydrochloride**

| Property | Physicochemical characteristics |
|---|---|
| Active | Trientine dihydrochloride |
| Synonyms | triethylenetetramine dihydrochloride, trientine hydrochloride (USP), trien, TETA |
| Chemical name | *N*,*N*'-Bis(2-aminoethyl)-1,2-ethanediamine dihydrochloride |
| CAS | 38260-01-4 |
| Formula | $C_6H_{18}N_4.2HCl$ |
| Structure |  |
| Molecular weight | 219.15 g/mol |
| Solubility | Freely soluble in water (>350mg/mL at pH 2-8). Soluble in methanol. Slightly soluble in alcohol. Insoluble in chloroform and in ether. |
| Log P | -1.8 |
| pKa | 9.33 |
| pH | 7.0-8.5 in solution |
| Source: https://pubchem.ncbi.nlm.nih.gov/compound/Trientine-hydrochloride | |

## 2.4.2.2 Pharmacodynamics of Trientine

Trientine is a copper-selective chelator that enhances systemic elimination of divalent copper by forming a stable complex that is readily excreted by the kidneys. Trientine is a chelator with a polyamine-like structure and copper is chelated by forming a stable complex with the four constituent nitrogens in a planar ring [Cufence, EPAR]. Trientine can lower blood and tissue copper levels and, when given chronically,

prevents copper accumulation and injury [Liver Tox, 2012]. Trientine may also chelate copper in the intestinal tract and so inhibit copper absorption [(SmPC, Cufence 200 mg hard capsules].

#### 2.4.2.2.1 Primary Pharmacodynamics

Trientine dihydrochloride (trientine) is an alternative long-term medicinal copper chelating agent for patients with WD who are intolerant to penicillamine [Sone H et al, 1996].

The disposition behaviours and de-coppering effect of triethylenetetramine dihydrochloride (trientine), a selective chelating agent for copper and an 'orphan drug' for Wilson's disease (WD), have been evaluated in various animal models [Iseki K et al, 1992]. Four animal models of WD have been established: the Long-Evans cinnamon (LEC) rat, the toxic-milk mouse, the Atp7b knockout mouse and the Labrador retriever. The existing models of WD all show good similarity to human hepatic WD and have been helpful in developing an improved understanding of the human disease [Reed E et al, 2018].

- **Increase in trientine urinary excretion of copper**

A study that compared the effect of trientine in LEC rats and Wilstar rats, suggested that both acceleration of urinary excretion of copper and reduction of hepatic copper levels were observed with treatment of trientine in LEC rats aged 6 weeks. In LEC rats aged 13 weeks, however, no de-coppering effect from the liver was observed, though urinary excretion of copper was increased. It was concluded that trientine has a pharmacological effect in disease state, especially in the early stages of hepatitis. Trientine was administered to the rats once a day for 7 days (25 mg/kg body weight) by gastric intubation [Iseki K et al, 1992].

- **Reduction in hepatic copper levels.**

In another study, the effects of trientine on the spontaneous development of hepatitis and hepatic tumours in LEC rats through the accumulation of copper in the liver were examined [Sone H et al, 1996]. Male LEC rats were given trientine in their drinking water at 1500 ppm for 18 weeks, from 6 weeks to 24 weeks of age in the short-term experiment, and 1500 ppm for 27 weeks and then 750 ppm for 52 weeks, from 8 to 87 weeks of age in the long-term experiment.

Development of hepatitis was observed in the control LEC rats at 18 weeks of age. Histological findings revealed that the short-term administration of trientine inhibited the development of hepatitis remarkably. Copper levels in the liver were decreased by a maximum of 50 percent. In the long-term administration of trientine, the incidence of hepatic cell carcinoma (HCC) in the treated rats was 67 percent that of the untreated LEC rats. The copper level in the liver of treated rats was reduced by 33 percent at 87 weeks of age. No effects on the levels of copper, iron, or zinc in the liver of Long-Evans Agouti (LEA) rats was detected, and no adverse effects were detected in either LEC or LEA rats after both short- and long-term administration of trientine in drinking water [Sone H et al, 1996].

- **Decrease in intestinal absorption of copper**

Copper is acquired from the diet by intestinal absorption and is subsequently distributed throughout the body. When in excess, copper's oxidative potential can induce free radical production and result in cellular damage. Tight regulation of copper homeostasis, maintained by mechanisms involving uptake, transport, storage, and excretion of copper, is therefore required. Severe imbalance of copper homeostasis can occur with some hereditary disorders of trientine not only increases urinary copper excretion, but also decreases intestinal copper absorption by 80% [Iseki K et al., 1992]. The decline of urinary excretion of trientine in LEC rats is thought to be due mainly to the lowering of the functional activity of the kidney, because urinary excretion of creatinine and phenolsulfonphthalein (PSP) were significantly lower in LEC rats than those in Wistar rats. Both acceleration of urinary excretion of copper and reduction of hepatic copper levels were observed with treatment of trientine in LEC rats aged 6 weeks. In LEC rats aged 13 weeks, however, no de-coppering effect from the liver was observed, though urinary excretion of copper was increased. These results suggest that trientine has a pharmacological effect in disease state, especially in the early stages of hepatitis [Iseki K et al., 1992].

### 2.4.2.2.2 Secondary Pharmacodynamics

Trientine has demonstrated several other pharmacodynamic actions beyond its copper-chelation property that has found its place in the treatment of WD. Some of these actions are summarised below.

- **Anti-cancer properties**

Trientine has been found to have anti-cancer properties. Various mechanisms proposed as possible modes of action of Trientine on cancer cells include: its telomerase inhibition action, by which trientine might have the selective inhibitory effect or cytotoxicity on tumour growth, as telomerase is an essential factor in cellular immortalization and tumorigenesis, which is expressed in over 85% of all human cancers; antiangiogenesis, as copper plays a key role in angiogenesis; promotion of apoptosis; and, induced expression of antioxidants through prolonged depletion of copper via trientine chelation [Lu J, 2010; Lixia G et al, 2008].

Trientine can also be used to overcome cisplatin resistance in ovarian cancer cells by decreasing the over-expressed Cu/Zn superoxide dismutase. Combination therapy using cisplatin and trientine could thus be a possible clinical entry point for trientine chemotherapy in cancer [Lu J, 2010].

In pre-clinical studies, trientine has been shown to be effectively inhibiting the growth of various tumours or tumour cells, including neuroblastoma, HCC, HeLa cells, colorectal carcinoma, and breast cancer cells (MCF-7). Long-term treatment with triethylene tetramine (TETA), at 50 or 100 μM, induced marked cellular senescence phenotypes accompanied by increased time of population doubling of MCF-7 cells [Lixia G et al, 2008].

Copper deficiency induces apoptosis in a variety of cells. Trientine, as a Cu-chelating agent, inhibits tumour growth in a murine transplantation model using fibrosarcoma and induces apoptosis in tumour cells *in vivo* and *in vitro* [Kadowaki S et al, 2009].

- **Usefulness in the treatment of diabetic mellitus and its complications**

Trientine has recently been identified as the first in a new class of anti-diabetic molecules. [Cooper GJ et al, 2011]. Significantly, long-term trientine treatment can restore the structure and function of organs damaged by diabetes without lowering blood glucose. Oral treatment with trientine or another chelator, citrate, prevented the development of cardiomyopathy in the Zucker diabetic rat, an animal model of type 2 diabetes mellitus. In particular, trientine treatment prevented cardiac dilatation, and also improved ejection fraction and myocardial relaxation. These data supported the fact that trientine could be used for the prevention of diabetic heart disease [Cooper GJ et al, 2011].

**Diabetic neuropathy**

There is evidence that trientine treatment can improve defects in motor nerve conduction velocity induced by diabetes. Impaired perfusion of nerve endoneurium is a major cause of nerve fiber dysfunction in experimental diabetes. Oxygen free radical activity is elevated in diabetes mellitus and has been implicated in the aetiology of vascular complications. *In-vitro* experiments suggest that autoxidation reactions of glucose, catalyzed by free transition metal ions, are a potential source of free radicals in diabetes [Cameron NE et al, 1995].

When it was investigated whether chronic treatment with deferoxamine and trientine, transition metal chelating agents which can prevent autoxidation, could correct nerve conduction and blood flow changes in streptozotocin-diabetic rats, a 20% reduction in sciatic nerve motor conduction velocity after 2 months diabetes was 90% ameliorated by 2 weeks of treatment with deferoxamine or trientine. Sciatic endoneurial nutritive blood flow was 45% reduced by diabetes, but was completely corrected by treatment. In contrast, transition metal chelation had no effect on blood flow or conduction velocity in nondiabetic rats. This suggests that impaired transition metal homeostasis plays an important role in elevated free radical generation. It is plausible that chelators like trientine could have a therapeutic role in the chronic neuropathic and micro/macrovascular changes in diabetic patients [Cameron NE et al, 1995].

**Diabetic nephropathy**

It has been observed that highly selective copper chelation restores cardiovascular structure and function damaged by diabetes. In a rat model, renal tissue copper was substantively elevated by diabetes and normalised by trientine treatment, which also suppressed whole-kidney and glomerular hypertrophy without lowering blood glucose levels. In contrast, although renal tissue zinc and iron levels were also modestly elevated by diabetes, neither was restored by trientine. The striking effects of trientine to improve renal structure and function in diabetes were probably mediated through its actions to normalise tissue copper. Creatinine ratio was significantly elevated in diabetic rats and lowered by trientine treatment [Cooper GJ et al, 2011; Gong D et al, 2009].

**Diabetic heart disease**

In diabetic animals with established left ventricular hypertrophy (LVH) and heart failure, 7 weeks oral trientine therapy significantly alleviated heart failure, improved

cardiomyocyte structure and reversed elevations in left ventricular (LV) and aortic collagen and $\beta_1$-integrin. It did this without lowering blood glucose or blood pressure [Cooper GJ et al, 2011].

In another study, oral treatment with trientine or another chelator, citrate, prevented the development of cardiomyopathy in the Zucker diabetic rat, an animal model of T2DM. In particular, trientine treatment prevented cardiac dilatation, and also improved ejection fraction and myocardial relaxation. These data were interpreted as supporting the use of trientine or citrate for the prevention of diabetic heart disease [Cooper GJ et al, 2011; Baynes JW et al, 2009].

- **Usefulness in the treatment of Alzheimer's disease**

Cerebrovascular amyloid angiopathy has emerged as one of the key pathogenetic processes in Alzheimer's disease (AD) and a similar process probably also makes a significant contribution in vascular dementia. There is mounting evidence that alterations in copper metabolism or combined dysregulation of copper and zinc may play an important role in the pathogenesis of AD [Bush AI et al, 2002].

### 2.4.2.3 Safety Pharmacology

No stand-alone safety pharmacology studies of trientine were identified. Safety pharmacology parameters of neurological examinations, electrocardiography, and blood pressure measurements, were reported by Maemura 1998 from repeated dose toxicity studies in dogs of 4- and 26- weeks study duration. No treatment-related changes in blood pressure or electrocardiogram measurements were observed. Neurological changes were observed at high dose levels, which recovered on cessation of treatment and were considered to be consistent with the effects of copper deficiency. Regarding safety pharmacology, published evidence from these repeated dose toxicity studies in dogs indicated no safety concerns [Cufence EPAR].

# 2.4.3 PHARMACOKINETICS

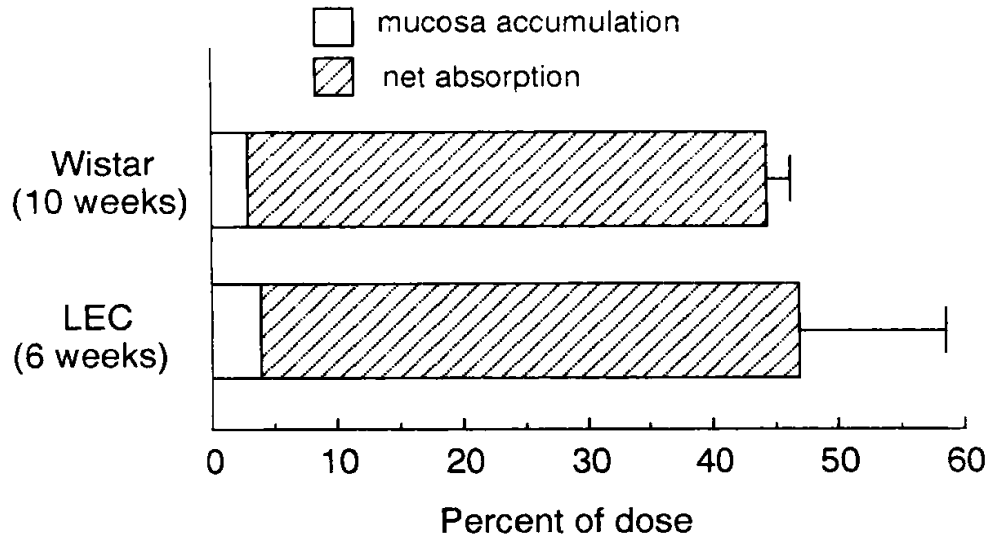
## 2.4.3.1 Absorption and Distribution

Trientine is mainly absorbed by the plasma membrane of intestinal epithelial cells, with low contribution of tight junction permeation. Several non-clinical studies show that oral bioavailability is strongly impaired by food intake. Oral bioavailability of trientine shows strong variability in the range of 5-25%, with relatively short half-life, as reported from literature. Autoradiographic data shows a maximum around 1 hour after administration, with high radioactivity mainly in the liver and kidney, with no evidence of retention in tissues after IV or oral administration [Cufence EPAR].

The absorption behaviour of trientine after oral administration was found to be very poor and variable in LEC rat model of Wilson's disease. An *in-situ* loop experiment was conducted to assess the absorption behaviour of trientine. The study results suggested percent of disappearance from the loop and accumulation in the mucosa in LEC rats were nearly the same as those of Wistar rats (Figure 1). Moreover, there were no significant differences in the time course of trientine plasma level during loop study between LEC and Wistar rats (Figure 2) [Iseki K, et al, 1992].

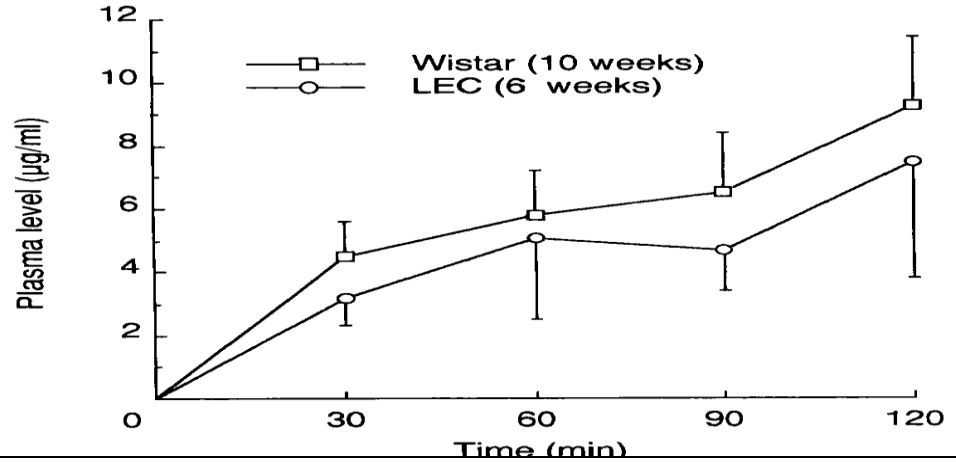
The intestinal absorption rate in normal male Wistar rats has been reported to be 42% in the jejunum and 22.5% in the ileum using an *in-situ* loop method. In Long-Evans Cinnamon (LEC) rats, the model organism for Wilson's disease, the jejunum absorption rate has been reported to be approximately 46% and without statistical significance when compared with data derived from Wistar rats. In Sprague Dawley rats, the extent of absorption after oral TETA administration has been reported to be 44.3% [Iseki K, et al, 1992].

**Figure 1. Absorption behaviours of trientine (25 mg kg$^{-1}$ body weight) from the jejunal loop of Wistar (10 weeks old) and LEC rats (6 weeks old) for 2 h** [Iseki K et al, 1992]




* **Results were expressed as the mean of three experiments with SD.**

**Figure 2. Time course of plasma concentration of trientine during the loop studies in Wistar (10 weeks old) and LEC rats (6 weeks old)** [Iseki K et al, 1992]




**Results were expressed as the mean of three experiments with SD.**

The absorption rate into the intestine, following oral trientine ingestion, appears to be relatively slow, probably due to its poor absorption from the intestine, where it is metabolized and inactivated. Only about 1% of the absorbed trientine and 8% of biotransformed acetyltrien are detectable in the urine [Rupp C et al, 2019].

Results obtained from rat and dog studies show that TETA has a relatively slow absorption and apparently incomplete intestinal absorption. The $T_{max}$ for rats, dogs and rabbits after oral TETA administration is 0.5-2 h**,** indicating an overall slow gut absorption [Lu J, 2010].

*In-vitro* studies have been carried out to determine the uptake characteristics of TETA by rat intestinal brush-border membrane vesicles. The mechanism of absorption is similar to those of physiological polyamines, such as spermine and spermidine, with respect to excessive accumulation in vesicles, pH dependency, temperature dependency and the ineffectiveness of $K^+$ diffusion potential. The initial uptake of TETA has a $K_m$ value of 1.13 mM, which is larger than that observed for spermine and spermidine. The uptake rate of TETA can be inhibited in a dose-dependent manner by spermine and spermidine [Tanabe R et al, 1996].

As summarised here by Lu J (2010), overall, the bioavailability of oral TETA administration is relatively low in rats and food intake seems to reduce it further: The bioavailability range of oral trientine in fasted rats was first reported at 6-18% (gibbs KR et al, 1986). Later reports provided similar results. One study (Kobayashi M et al, 1990) reported a bioavailability of 2.31% in non-fasted rats and 6.56% in fasted rats. A second report (Miyazaki K et al, 1990) showed bioavailability in three fasted rats at 5.6%, 5.7% and 16.4%, respectively. A third report (Takeda S et al, 1995) provided a bioavailability of 14.0% in non-fasted rats and 25.5% in fasted rats. A fourth report (Tanabe R, 1996) determined that the bioavailability in fasted rats was 13.78% [Lu J, 2010].

TETA is widely distributed into various tissues in rats, either in the form of unchanged parent compound or bio transformed metabolite. The apparent volume of

distribution is consistent with trientine being widely distributed. The study using $^{14}C$ radio labelled trientine showed that TETA could be found in most rat tissues, including cerebrum, cerebellum, hypophysis, eyeball, harderian gland, thyroid, submaxillary gland, lymphatic gland, thymus, heart, lung, liver, kidney, adrenal, spleen, pancreas, fat, brown fat, muscle, skin, bone marrow, testis, epididymis, prostate gland, stomach, small intestine and large intestine [Lu J, 2010]. In the analyses, it was observed that both the parent compound and metabolite(s) exist in all tissues [Lu J, 2010]. A later report (Tanabe R, 1996) confirmed such findings, showing that concentration ratios of liver/plasma and kidney/plasma were greater than 1, while brain, lung, spleen and white fat have ratios lower than 1 [Lu J, 2010]. It is proposed that TETA shares a common transport mechanism with polyamines in intestinal uptake. It is likely that TETA is also transported across biological membrane into mammalian cells by the same transporter for polyamines. It is therefore not surprising that TETA is widely distributed in the body and can be accumulated in the tissues [Lu J, 2010].

Carefully designed study in normal Sprague-Dawley rats showed that D-penicillamine gets into liver tissue and mobilizes copper whereas trientine competes better than D-penicillamine for copper bound to albumin in the plasma compartment [Roberts EA, 2019].

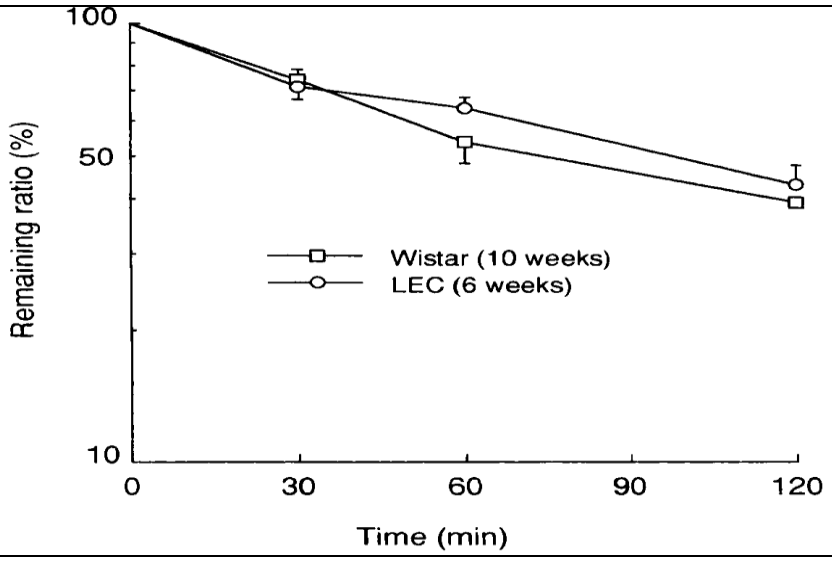
### 2.4.3.2 Metabolism

The predominant metabolite in rat was N1-acetyltriethylenetetramine (MAT), which was also found in the urine of treated subjects, besides trientine, N1, N10-diacetyltriethylenetetramine (DAT) and three minor metabolites. SSAT2 (thialysine acetyltransferase) was shown to be the main metabolizing enzyme responsible for low oral bioavailability [Cufence EPAR].

Trientine appears to undergo extensive first-pass metabolism after intestinal absorption. Kodama and colleagues identified the main metabolite of trientine as acetyl-trientine in humans and showed that it had little chelating activity [Kodama H et al, 1997].

*In-vitro* trientine metabolism results in the S9 fraction of the liver are illustrated in Figure 3. In both LEC and Wistar rats, about 50 per cent of the trientine was eliminated from the reaction system during the 2 h. The metabolic rates in LEC rats were nearly the same as those of Wistar rats [Iseki K et al, 1992].

**Figure 3. Elimination of trientine in the S9 fraction of the liver homogenates of Wistar (10 weeks old) and LEC rats (6 weeks old)** [Iseki K et al, 1992]




**Results were expressed as the mean of three experiments with SD.**

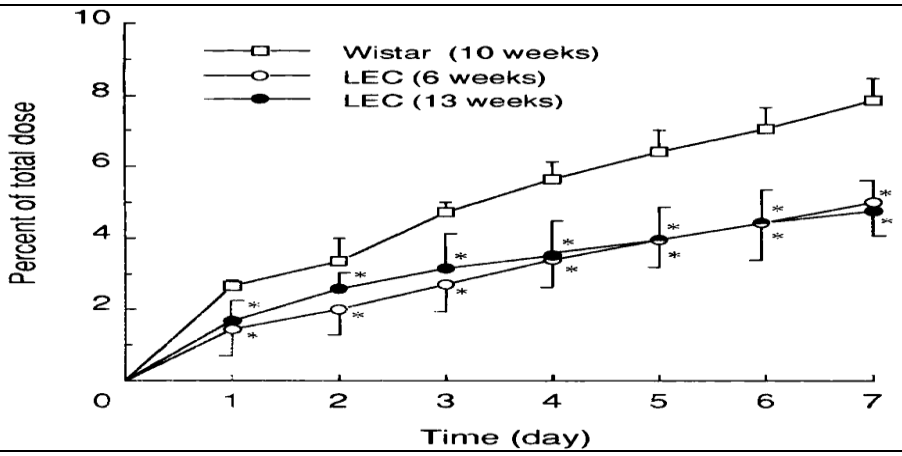
*In-vitro* experiments have shown that about 50% of TETA was eliminated from the S9 liver fraction system after 2 hr of incubation. One *in-vivo* study in rats (Kobayashi M et al, 1990) showed that after oral administration of trientine, only 3.1% of the dose was found in the 24-hr urine collection as the unchanged parent compound, while metabolites accounted for 32.6% of the oral dose [Lu J, 2010]. Another *in-vivo* study (Takeda S et al, 1995) reported (that 2.6% of the dose was recovered from 24-h urine collection as the unchanged parent compound, and 11% metabolites. TETA metabolite levels in rat tissues have been investigated in two studies [Lu J, 2010]. In one study Tanabe R, 1996), after oral administration of trientine, the plasma $AUC_{0\text{-}6}$ hr of the metabolite MAT has been reported to be higher than that of unchanged TETA in rats [Lu J, 2010]. The same report and another early report (Takeda S et al, 1995), both showed that MAT existed in rat tissues at similar levels observed for the unchanged parent compound [Lu J, 2010].

### 2.4.3.3 Elimination

In LEC rats, urinary excretion of trientine was remarkably lower than that in Wistar rats. The absorption rates from the jejunal loop and *in-vitro* metabolism in the liver S9 fraction were approximately the same for both strains. The decline of urinary excretion of trientine in LEC rats was thought to be due mainly to the lowering of the functional activity of the kidney, because urinary excretion of creatinine and PSP were significantly lower in LEC rats than those in Wistar rats. Both acceleration of urinary excretion of copper and reduction of hepatic copper levels were observed with treatment of trientine in LEC rats aged 6 weeks. In LEC rats aged 13 weeks, however, no de-coppering effect from the liver was observed, though urinary excretion of copper was increased [Iseki K et al, 1992].

The cumulative urinary excretions of total trientine following multiple p.o. doses (25 $mg/kg^{-1}$ for 7 days, once per day) are illustrated in Figure 4.

**Figure 4. Cumulative urinary excretion of total trientine after oral administration (25 mg kg-$^{1}$ day-$^{1}$) for 7 days in Wistar (10 weeks old) and LEC rats (6 and 13 weeks old)** [Iseki K et al, 1992]




**Results were expressed as the mean of three to four experiments with SD; *p<0-05 from values of Wistar rats.**

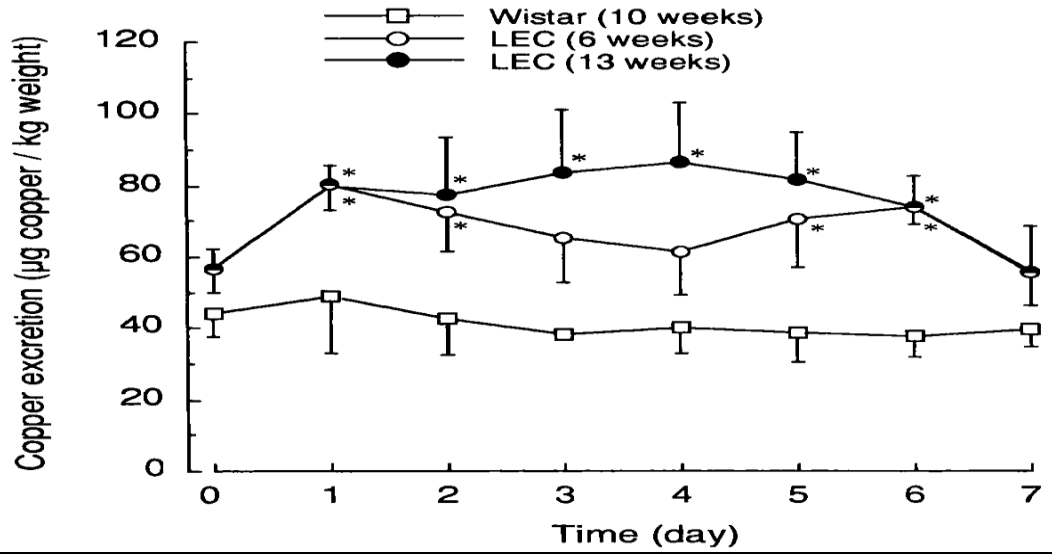
Comparative data concerning the renal function of LEC and Wistar rats are shown in Table 2. In LEC rats, urinary excretion of creatinine and PSP were significantly lower than those of Wistar rats. Amount of excreted copper in each 24 h urine collection period of LEC and Wistar rats following multiple p.o. doses of trientine are illustrated in Figure 4. The data of non-treated rats (24 h-period urine) are indicated at Day 0 in Figure 5. It was found that urinary excretion of copper in LEC rats was greater than that of Wistar rats and was accelerated significantly with the treatment of trientine, especially in 13-week-old rats. In Wistar rats, on the contrary, the accelerating effect of trientine was not observed throughout the experimental period [Iseki K et al, 1992].

**Table2. Comparison of kidney function between Wistar (aged 10 weeks) and LEC rats (Aged 6 weeks)** [Iseki K et al, 1992]

| | **Urinary PSP excretion (percent of dose per hour)** | **Urinary creatinine excretion (mg day kg$^{-1}$ weight)** |
|---|---|---|
| Wistar (10 weeks) | 52.0 ± 4.9 | 36.2±0.4 |
| LEC (6 weeks) | 1.8 ± 2.6* | 29.3 ± 0.6** |

Results were expressed as the mean of 3-5 experiments with SD
*Significantly different from Wistar, p<0*00l.
**$p < 0.05$.

**Figure 5. Comparison of urinary excretion of copper between Wistar (10 weeks old) and LEC rats (6 and 13 weeks old) after trientine administration** [Iseki K et al, 1992]




Results were expressed as the mean of three to four experiments with SD; *p <0.05 from the values of day zero before trientine administration.

Most of the absorbed TETA is excreted via urine as bile and lung excretions appear to be minimal in animal studies [Lu J, 2010]. One study (Takeda S et al, 1995) found that after oral trientine administration to rats, 0.69% of the dose was found in expired air and 0.86% of the dose was excreted via bile [Lu J, 2010]. The urinary excreted TETA is mainly in the form of acetylated metabolites, while the unchanged parent compound represents a smaller percentage of the dose. The renal clearance of TETA in rat is about 30% higher than creatinine clearance, which indicates TETA is actively excreted from the renal tubule into urine [Lu J, 2010]. TETA metabolites, MAT and DAT, are also straight-chain structures, and with 4 amino groups, they should be able to be actively excreted in kidney as well. Therefore, it is not surprising that a large amount of metabolites are found in rat urine. Diseases that compromise kidney function in rats seem to affect urinary excretion of TETA [Lu J, 2010]. One early study (Iseki K et al, 1992) reported that LEC rats, a rat model of Wilson's disease, had significant lower urinary TETA excretion than that in normal Wistar rats [Lu J, 2010]. This was due to the impairment of kidney function in LEC rats. The plasma elimination half-lives ($T_{1/2}$) of TETA in rat, dog and rabbit are between 0.5 – 2 h. This suggests that TETA is quickly removed from the blood [Lu J, 2010].

### 2.4.3.4 Pharmacokinetic Drug interactions

There is no interaction between trientine and the substrate of the H+/organic cation antiporter or aminoglycoside antibiotics. A specific transport system (Na+/spermine antiporter on the renal brush-border membrane) is responsible for renal excretion of trientine (but does not recognize the trientine-copper complex). Drugs that change the concentration of sodium ions in renal proximal tubules, such as diuretics, can increase the trientine excretion since the increase in the luminal concentration of sodium ions accelerates the Na+/spermine antiporter. The low protein binding properties of trientine make drug interactions as a consequence of displacement of trientine from plasma protein binding unlikely [Cufence, EPAR].

Diuretics drugs such as furosemide increase the excretion of Na+ ions in the urine therefore, it is probable that the increase in Na+ concentration in urine produced by diuretics drug affects the activity of carrier-mediated trientine secretion [Kobayashi M et al, 1999). To confirm this point various diuretics drugs were co-administered with trientine to rats. All the experiments were performed on 3-5 male Wistar rat (250-300 g). TETA saline solution was injected into jugular vein at dose of 2.5 mg/kg. Urine was collected from the bladder at 0.5, 1, 2, 3 and 4 hr after administration of drugs. As shown below in Table 3, acetazolamide (ACZ) and furosemide (FSM) significantly increase the urinary secretion of trientine, while trichlormethiazide (TCM) had no effect [Kobayashi M et al, 1999].

**Table 3. Effect of various drugs on cumulative urinary excretion of trientine after intravenous injection** [Kobayashi M et al, 1999]

| **Co-administered drug** | **Time (Hr)** | | | | |
|---|---|---|---|---|---|
| | **0.5** | **1** | **2** | **3** | **4** |
| No additive | 32.6 ± 1.7 | 57.4 ± 1.7 | 68.7 ± 2.1 | 71.2 ± 2.1 | 72.0 ± 2.1 |
| Spermidine | 34.7 ± 3.3 | 56.0 ± 2.7 | 70.0 ± 2.0 | 71.4 ± 1.1 | 72.8 ± 1.8 |
| Spermine | 30.5 ± 1.1 | 52.4 ± 2.5** | 62.7 ± 3.4* | 65.5 ± 2.2** | 66.3 ± 2.1** |
| Cimetidine | 30.0 ± 2.9 | 58.0 ± 4.4 | 69.9 ± 2.0 | 70.7 ± 2.0 | 71.4 ± 2.5 |
| Tobramicin | 30.0 ± 2.0 | 53.3 ± 2.6 | 68.3 ± 0.7 | 71.4 ± 1.9 | 72.7 ± 1.4 |
| Amikacin | 30.5 ± 3.3 | 57.0 ± 3.7 | 71.4 ± 2.1 | 74.6 ± 2.3 | 74.9 ± 1.7 |
| CuSO4 | 26.9 ± 2.0** | 50.1 ± 3.6** | 58.1 ± 3.6** | 61.2 ± 4.9** | 62.8 ± 4.2** |
| Acetazolamide | 49.4 ± 4.6** | 89.4 ± 10.5** | 121.4 ±14.4** | 139.4 ± 12.1** | 144.4 ± 13.0** |
| Furosemide | 42.9 ± 1.6** | 42.9 ± 5.8** | 86.1 ± 4.7** | 88.9 ± 4.8** | 89.1 ± 3.9** |
| Trichlormethiazide | 35.1 ±3.9 | 55.3 ± 3.4 | 64.5 ± 5.2 | 70.5 ± 1.9 | 72.6 ± 1.8 |

Note: Trientine • 2HCI (2.5 mg/kg) was administered by intravenous injection with or without spermidine (2.5 mg/kg), spermine (2.5 mg/kg}, cimetidine (3.3 mg/kg), tobramicin (1.2 mg/kg), amikacin (1.5mg/kg), CuSO4 • 5H20 (2.8 mg/kg), acetazolamide (8 mg/kg), furosemide (0.3 mg/kg) and trichlormethiazide (0.1 mg/kg). Each value represents the mean ± SD of 3 to 5 rats.
* Significantly different from the value of "No additive" at P<0.05, **P<0.01.

# 2.4.4 TOXICOLOGY

### 2.4.4.1 Acute toxicity studies

Trientine dihydrochloride is a known irritant, especially to mucus membranes, upper respiratory tract and skin, and induces skin sensitisation in guinea pigs, mice and man [Cufence, EPAR].

Based on the literature, trientine is considered to have moderate acute toxicity after oral administration in animals ($LD_{50}$ rat >2000 mg/kg birth weight) and moderate toxicity after dermal application ($LD_{50}$ rabbit 550-805 mg/kg bw). The non-clinical data reveal no special hazard for humans based on a series of studies investigating cardiovascular safety pharmacology, repeated dose toxicity, genotoxicity and toxicity to embryofoetal development [Cufence, EPAR].

### 2.4.4.2 Sub-chronic toxicity studies

Greenman DL et al (1996) investigated the sub-chronic toxicity of triethylenetetramine dihydrochloride B6C3F1 in mice and F344 rats. Mice and rats received trien-2HCl in the drinking water at concentrations of 0, 120, 600, or 3000 ppm for up to 92 days. Trien-2HCl toxicity occurred only in mice in the highest dose group fed an AIN-76A diet. Increased frequencies of inflammation of the lung interstitium and liver periportal fatty infiltration were seen in both the sexes. Kidney and body weight were reduced in males, as was the incidence of renal cytoplasmic vacuolization [Greenman DL et al, 1996].

Similarly, Yanagisawa T et al (1998) studied the possible sub-chronic and chronic toxicity of triethylenetetramine dihydrochloride when administered orally to male and female F344 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 and also evaluated the reversibility of any effect [Yanagisawa T et al, 1998].

Dosing was started at 33 to 42 days of age. The duration of the 4- or 8-week study was intended initially for 4 weeks in F-344 rats. 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 [Yanagisawa T et al, 1998].

In the 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 phosphatise activities evident in animals receiving 350 or 1200 mg/kg/day. After 4 or 8 weeks, and during 8 weeks of treatment, high lung weights

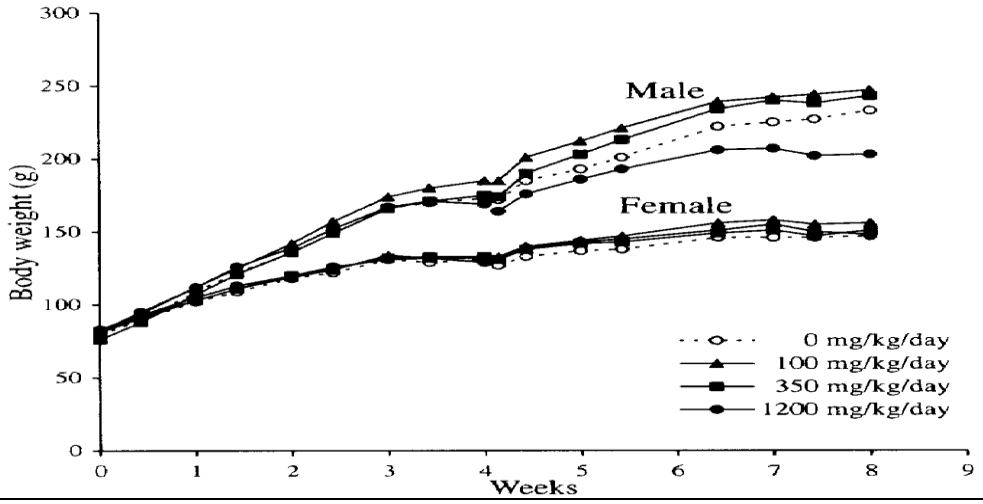
and bronchiolar epithelium hypertrophy and broncho-alveolar pneumonia were recorded for animals receiving 1200 mg/kg/day, and submucosal acute inflammation within the glandular region of the stomach was recorded for males receiving 350 or 1200 mg/kg/day and in all treated female groups [Yanagisawa T et al, 1998].

The experimental design and the mean body weight findings in the 4 to 8-week study are presented in Table 4 and Figure 6.

**Table 4. Experimental design of 4- to 8- weeks studies of Trientine 2HCl** [Yanagisawa T et al, 1998]

| 4- or 8- weeks study | | |
|---|---|---|
| **Dosage** | **No. of animals** | |
| **mg/kg/day** | **4 weeks** | **8 weeks** |
| 0 | 5 | 5 |
| 100 | 5 | 5 |
| 350 | 5 | 5 |
| 1200 | 5 | 5 |

**Figure 6. Mean body weight of rats in 4- or 8-week toxicity study of trientine-2HCl** [Yanagisawa T et al, 1998]




### 2.4.4.3 Chronic toxicity studies

In the 26-week rat study, reversibility was determined from 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). Dosing was started at 33 to 42 days of age [Yanagisawa T et al, 1998].

In the 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

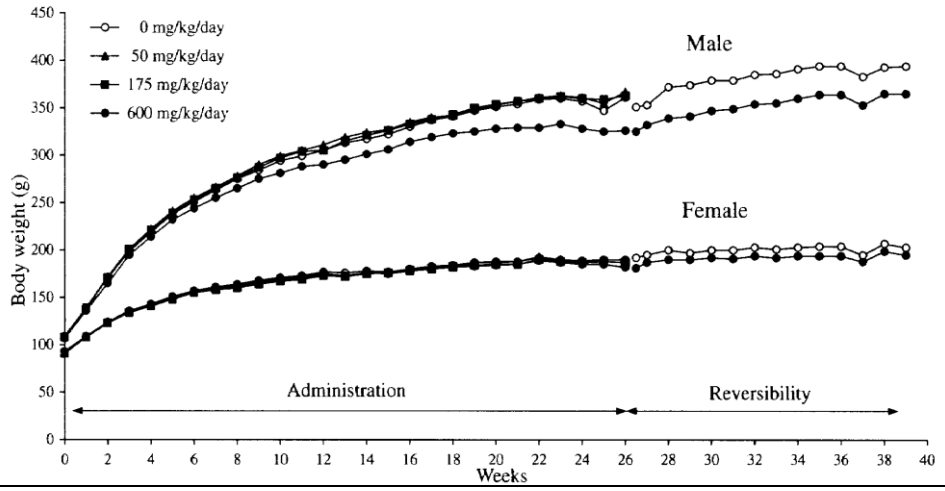
examinations showed changes similar to those indicated in the 4- or 8-week study. The low plasma copper concentrations seen in males receiving 600 mg/kg/day, the slightly low liver copper concentrations found in animals receiving 600 or 175 mg/kg/day and the high urinary copper concentrations found in all treated groups, were attributed to the phamacological 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 [Yanagisawa T et al, 1998].

The experimental design and the mean body weight findings in the 26-week study are presented in Table 5 and Figure 7.

**Table 5. Experimental design of 26 weeks studies of Trientine 2HCL [Yanagisawa T et al, 1998]**

| 26-weeks study | | |
|---|---|---|
| **Dosage** | **No. of animals** | |
| **mg/kg/day** | **Main study** | **Reversibility study** |
| 0 | 12 | 8 |
| 50 | 12 | 0 |
| 175 | 12 | 0 |
| 600 | 12 | 8 |

**Figure 7. Mean body weight of rats in 26-week toxicity study of trientine-2HCl** [Yanagisawa T et al, 1998]




It was concluded from these studies 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 less than 50 mg/kg/day for males [Yanagisawa T et al, 1998]. NOAEL in dogs was established to be 50 mg/kg/day in a 26 week (oral administration) study,

associated with a mean $C_{max}$ and AUC of approximately 22 µg/mL and 73 µg.h/mL, respectively – presenting an adequate margin of exposure [Cufence, EPAR].

### 2.4.4.4 Genotoxicity Study

The OECD SIDS triethylenetetramine 2002 classifies the genotoxic profile of trientine as low priority/concern. Trientine shows mutagenicity in bacterial cells with and without S9 fraction in a broad range of bacterial strains, which could indicate that this is not caused solely by oxidative stress (triggered as a consequence of copper depletion). Supplementation with $CuCl_2$ showed reduction or elimination of mutagenic effects, but is confounded by the bactericidal effects of copper. Oxidative stress also has an impact on tests in cultured mammalian cells, where contradictory results were observed with trientine. Published results of mouse micronucleus tests after oral or intra-peritoneal administration examining the bone marrow or peripheral blood were negative, but had some deficiencies in the protocol [Cufence, EPAR].

Effects of treatment with trientine, a specific copper-chelating agent, on accumulation of copper and induction of DNA strand breaks were investigated in Long–Evans Cinnamon (LEC) rats, an animal model for human Wilson's disease. When LEC rats were treated with trientine from 10 weeks of age, hepatic copper contents did not increase and were maintained at the same levels as those in 10-week-old LEC rats. When the amounts of DNA single-strand breaks (SSBs) were estimated by a comet assay, SSBs of DNA were induced in a substantial population of LEC rat hepatic cells around 8 weeks of age and the amounts of SSBs increased in an age-dependent manner from 8 to 15 weeks of age. When LEC rats were treated with trientine from 10 weeks of age, the observed number of cells with DNA damage decreased dramatically, suggesting that induction of SSBs of DNA was inhibited and/or SSBs were repaired during the period of treatment with trientine. The results show that treatment of LEC rats with trientine decreases the number of DNA strand breaks observed, although copper contents remain high in the liver [Hayashi M et al, 2004].

Copper is accumulated in the kidneys of LEC rats in an age-dependent manner from 12 to 18 weeks of age. When LEC rats were treated with trientine from 10 weeks of age, renal copper contents did not increase and were maintained at the same levels as those in 4-week-old LEC rats. Estimation of the amounts of DNA single-strand breaks (SSBs) by comet assay showed that SSBs of DNA were induced in a substantial population of LEC rat renal cortex cells around 12 weeks of age and that the amounts of SSBs increased in an age-dependent manner from 12 to 18 weeks of age. When LEC rats were treated with trientine from 10 weeks of age, the observed number of cells with DNA damage decreased, suggesting that induction of SSBs of DNA was inhibited and/or SSBs were repaired during the period of treatment with trientine. The results show that SSBs of DNA in LEC rat kidney cells are induced prior to occurrence of clinical signs of hepatic injury and that treatment of LEC rats with trientine decreases the number of DNA strand breaks [Hayashi M et al, 2004].

The LEC rat is widely accepted as a rodent model of Wilson disease and presents lots of common clinical features with human WD [Huster D, 2019]. The rat Atp7b gene for Atp7b was cloned by Wu et al. in 1994 [Huster D, 2019]. The gene defect was identified as a partial deletion of at least 900bp of the coding region at the 30 end and at least 400bp of the downstream untranslated region. Further genetic experiments revealed the autosomal recessive pattern of this condition [Huster D, 2019].

With respect to the occurrence of liver cancer after hepatitis and jaundice, DNA adducts formed either directly by hydroxyl radicals or via reactive aldehydes originating from lipid peroxidation have amply been demonstrated in WD rats. This genotoxicity may explain a late developing liver cancer in LEC rats, which is, however, very rare in WD patients [Zischka H et al, 2019].

### 2.4.4.5 Carcinogenicity

Only dermal, but no oral carcinogenicity studies with trientine in rodents are reported. Standard 2-year rodent bioassays in healthy rodents would anyhow have limited impact on the overall carcinogenicity risk assessment, based on confounding primary pharmacology of the compound. Importantly, LEC rats showed reduced levels of single strand breaks after treatment with trientine, indicating a reduction of endogenous DNA damage in a WD model. Thus it can be agreed, that separate carcinogenicity studies are not needed for trientine [Cufence, EPAR].

Angiogenesis is now recognized as a crucial process in tumour development, including hepatocellular carcinoma (HCC). Since HCC is known as a hypervascular tumour, antiangiogenesis is a promising approach to inhibit the HCC development [Yoshi Jet al, 2001]. In one study, the effect of Cu-chelating agents on tumour development and angiogenesis was examined in the murine HCCxenograft model created in 80 female 5-week-old BALB/c mice [Yoshi Jet al, 2001].

Although both trientine and penicillamine in the drinking water suppressed the tumour development, trientine exerted a more potent inhibitory effect than penicillamine. In combination with a Cu-deficient diet, both trientine and penicillamine almost abolished the HCC development. Trientine treatment resulted in a marked suppression of neovascularization and increase of apoptosis in the tumour, whereas tumour cell proliferation itself was not altered. *In-vitro* studies also exhibited that trientine is not cytotoxic for the tumour cells. On the other hand, it significantly suppressed the endothelial cell proliferation. These results suggested that Cu plays a pivotal role in tumour development and angiogenesis in the murine HCC cells, and Cuchelators, especially trientine, could inhibit angiogenesis and enhance apoptosis in the tumour with consequent suppression of the tumour growth *in-vivo*. Since trientine is already used in clinical practice without any serious side effects as compared to penicillamine, it may be an effective new strategy for future HCC therapy [Yoshi Jet al, 2001].

### 2.4.4.6 Reproductive and developmental toxicity

No fertility data are available but oestrous cyclicity was unaffected and reproductive organs were not identified as target organs in general repeat-dose toxicity studies in animals [Cufence, EPAR].

In pregnant animals, high-dose trientine associated with significant reductions in serum copper revealed an early effect on embryo survival and a marginally lower foetal weight. There was no evidence of embryo-foetal toxicity at lower dose levels despite dose-related reductions in serum copper. These effects were observed only at exposures sufficiently in excess of maximum human exposure to indicate little relevance to clinical use [Cufence, EPAR].

No teratogenic effects were observed in rabbits, while rats showed foetal abnormalities in the high dose group, significantly reduced after copper supplementation. In a dose-range finding study in pregnant rats conducted by the Applicant at lower doses, despite a dose dependent reduction in serum copper levels, there was no evidence of teratogenicity and the NOAEL was considered to be 750 mg/kg/day. Nevertheless, there was evidence of an early effect of treatment on embryo survival, which is not surprising as the impact of copper deficiency on the developing foetus is well established: different new-born copper-deficient species show similar abnormalities as known with swayback (enzootic ataxia) in lambs. Positive teratogenicity signals observed after prenatal exposure of mice, for instance, still leave it open if this represents an intrinsic property of the compound or a consequence of copper deficiency. Published experience with the use of trientine in pregnant women does not indicate major birth defects - but is too limited to make final conclusions. But clear evidence is given for the increased clinical risk of spontaneous abortions in untreated WD [Cufence, EPAR].

In another study, Sprague-Dawley rats were administered throughout pregnancy a complete purified control diet (containing 5 parts per million copper) or the same diet with added TETA at 0.17, 0.83, or 1.66%. These levels of TETA were comparable with dosages used clinically. At term, day 21 of pregnancy, foetuses were removed, and maternal tissues and foetuses were analysed. The pregnant rats in all groups appeared healthy throughout the experiment. With increasing amounts of TETA in the diet, the frequencies of both resorptions and foetal abnormalities increased (Table 6). Abnormalities primarily seen were massive haemorrhaging and oedema. TETA also had a pronounced effect on tissue copper and zinc concentrations. Maternal plasma copper concentrations (µg/dl) decreased with increasing amounts of drug fed from 127 (control) to 91, 45, and 5 for 0.17%, 0.83%, and 1.66% respectively. Copper levels were similarly affected in foetal liver; with increasing amounts of drug in the diet, foetal liver copper decreased (Table 6). The concentration of zinc in foetal liver was also affected by TETA; however, in contrast to copper, zinc concentration increased with increasing drug dosage. A similar effect of TETA on zinc concentration was also observed in maternal kidney.

These data show that the drug TETA is teratogenic in rats a dosages similar to those used clinically and that the teratogenicity may be due to the effects of TETA on copper and zinc metabolism [Keen CL et al, 1982].

**Table 6. Effect of Triethylenetetramine on Foetal Outcome: Mean & Plus ± SEM** [Keen CL et al, 1982]

| Added TETA* | Resorptions (%) | Abnormal foetuses | Foetal Liver Conc. (µg/g wet weight) of | |
|---|---|---|---|---|
| | | | Copper | Zinc |
| 0 (n = 7) | 0 | 0 | 15.3 ± 1.2 | 711.6 ± 4.3 |
| 0.17 % (n = 5) | 6 | 0 | 9.4 ± 1.8 + | 118.2 ± 9.3++ |
| 0.83 % (n = 9) | 9 | 23 | 5.1 ± 1.0++ | 168.5 ± 6.7++ |
| 1.66 % (n = 5) | 19 | 100 | 0.25 ± 0.17++ | 196.3 ± 12.3++ |

*n = no. of litters. Significantly different from control (student's t test): + $p<0.05$, ++ $p<0.01$.

### 2.4.4.7 Characterisation of Impurities

Trientine Dihydrochlorie Capsules 300 mg, comply with a relevant pharmacopoeia (USP in the absence of a Ph. Eur. monograph) limits for impurities, together with current versions of ICH Q3A for related substance, and Q3C for solvents, respectively, as discussed in section 3.2.P.5.5 that refers to section 3.2.S.5 for details (referencing further to section 3.2.S.4.5. for justifications). Based on the quality documentation and findings, there is no issue with unqualified chemical/organic impurities for this product.

Trientine Dihydrochlorie Capsules 300 mg, also comply with the requirements established for control of elemental impurities described in ICH Q3D guidelines, because the levels of elemental impurities are found below their PDE value & threshold limit of 30% of MPC: the levels are controlled within acceptable limits and there is no risk (associated to elemental impurities) for the patients that take these drug products according to their recommended posology and method of administration. All the observed elemental (metal) impurities level is meeting the 30% limit (ICH Q3D) oral limit, no further control required. For details with respect to impurity profile results, detailed information is available in section 3.2.P.5.5.

## 2.4.5 INTEGRATED OVERVIEW AND CONCLUSIONS

Trientine is a well-established active substance that has been authorised in the United Kingdom (UK) since 1985 for the treatment of WD in patients who are intolerant of penicillamine, and a number of generic products have been registered in the US, EU and the UK since then. The clinical experience to date has not indicated any cause for concern in terms of its efficacy and safety. The nonclinical evidences presented in this document are expected to provide further support to the marketing authorisation application of Trientine 200 mg hard capsules, manufactured by Waymade Plc, UK, as the generic version of the reference product, Cufence 200 mg hard capsules, manufactured by Univar BV, The Netherlands (EU/1/19/1365/001).

Trientine is a copper-selective chelator that enhances systemic elimination of divalent copper by forming a stable complex that is readily excreted by the kidneys. Trientine is a chelator with a polyamine-like structure and copper is chelated by forming a stable complex with the four constituent nitrogens in a planar ring.

This non-clinical overview of trientine summarises *in-vitro* and *in-vivo* studies, efficacy and pharmacodynamics studies in animal models from the published literature. It provides a comprehensive overview of published information on the pharmacodynamics and pharmacokinetics of trientine, including parameters in different animal species, together with a summary of public domain toxicology data.

The pharmacology studies sourced from published literature and included in this application demonstrate proof of concept for trientine chelation of copper in animal models, through both increase of urinary copper excretion, and decrease in intestinal copper absorption. The results are consistent with current understanding of the pharmacology of the product, and support the clinical aspects of this submission.

The disposition behaviours and de-coppering effect of triethylenetetramine dihydrochloride (trientine), as a selective chelating agent for copper in WD, have been evaluated in an animal model, Long-Evans Cinnamon (LEC) rats, and normal rats (Wistar). No de-coppering effect from the liver was observed, though urinary excretion of copper was increased. Results suggest that trientine has a pharmacological effect in disease state, especially in the early stages of hepatitis, supporting its use in WD.

Secondary pharmacology studies published for trientine at the non-clinical level relate to anti-cancer activity, Alzheimer´s disease, diabetic kidney- and cardiovascular disease, together with a potential chelating effect of other divalent cations, i.e. zinc and iron. This is not considered to be required for this application, with the effect of trientine in these areas most likely driven by its copper-chelating properties and not a consequence of off-target secondary effects.

Safety pharmacology aspects have been covered by clinical assessments of behaviour, respiration and cardiac safety performed during repeat-dose toxicity for the reference product – for which no findings were reported. Animal safety pharmacology is mainly intended to support benefit/risk assessment (and to avoid unpredicted side effects) during clinical development of new products, and being a generic version of an

already marketed product, it may entirely be superseded by clinical safety data for the reference product.

The pharmacokinetics study results obtained from rat and dog studies show that TETA has a relatively slow absorption and apparently incomplete intestinal absorption. The $T_{max}$ for rats, dogs and rabbits after oral TETA administration is 0.5-2 hours, indicating an overall slow gut absorption. TETA is widely distributed into various tissues in rats, either in the form of unchanged parent compound or bio transformed metabolite. The study using $^{14}C$ radiolabelled trientine showed that TETA could be found in most rat tissues, including cerebrum, cerebellum, hypophysis, eyeball, harderian gland, thyroid, submaxillary gland, lymphatic gland, thymus, heart, lung, liver, kidney, adrenal, spleen, pancreas, fat, brown fat, muscle, skin, bone marrow, testis, epididymis, prostate gland, stomach, small intestine and large intestine.

TETA is also transported across biological membrane into mammalian cells by the same transporter for polyamines. It is therefore not surprising that TETA is widely distributed in the body and can be accumulated in the tissues. *In-vitro* trientine is metabolised by the S9 fraction of the liver. About 50 per cent of the trientine was eliminated from the reaction system during the 2 hours. Most of the absorbed TETA is excreted via urine as bile and lung excretions appear to be minimal in animal studies.

Pharmacodynamic investigations showed no interaction between trientine and the substrate of the H+/organic cation antiporter or aminoglycoside antibiotics. A specific transport system (Na+/spermine antiporter on the renal brush-border membrane) was demonstrated to be responsible for renal excretion of trientine. Drugs that change the concentration of sodium ions in renal proximal tubules, such as diuretics, can increase the trientine excretion. The low protein binding properties of trientine make drug interactions as a consequence of displacement of trientine from plasma protein binding unlikely.

The single dose toxicity reported in the studies in this submission, of LD50 rat > 2000 mg/kg bw, was confirmed in an OECD-SIDS assessment report (OECD), that considered trientine of low acute toxicity after oral administration, and moderate toxicity after dermal application. For repeat dose toxicity assessments the duration, dosing, and use of at least two species of mammals, of which one was a non-rodent (dog) model, met requirements for adequate assessment. The main toxicological findings were consistent across different species.

Genotoxicity results from the *in-vitro* and *in-vivo* study included were consistent with the OECD SIDS triethylenetetramine 2002 report, which classifies the genotoxic profile of trientine as low priority and concern.

The toxicological studies reviewed and conclusions made in this submission are consistent with those in recent publications and reviews on this topic, with no new areas of concern presented that have not been previously considered in reviews of similar products. In particular, we found no new information that would have a bearing on the benefit/risk to patients for intended clinical use. The effects seen were largely consistent with induced copper deficiency in the plasma and liver of

previously copper normative animals and as such could be attributed to the pharmacological action of trientine.

The available pre-clinical information therefore suggests that the use of trientine for the treatment of WD in patients who are intolerant of penicillamine is safe and efficacious and it poses no undue hazard for humans when used in accordance with the information provided in the SmPC.

## 2.4.6 LIST OF LITERATURE REFERENCES

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