SCIENTIFIC DISCUSSION

This module reflects the initial scientific discussion for the approval of Wilzin. For information on changes after approval please refer to module 8.

1. Introduction

Wilzin contains zinc acetate dihydrate as active substance. With the present application, the applicant sought a marketing authorisation in the following indication: "Maintenance treatment of patients with Wilson's disease. Wilzin can be administered in symptomatic patients after initial decoppering therapy with a chelating agent or from the beginning in presymptomatic patients."

Wilson's Disease

Wilson's disease (also known as hepatolenticular degeneration) is an inherited autosomal recessive disorder characterised by the accumulation and toxicity of copper in various tissues.
Copper is an essential trace element and an important metal co-factor for many enzymes. Normally, excess dietary copper is bound in the hepatocyte by cytoplasmic proteins, particularly metallothioneins (cysteine-rich intracellular proteins capable of binding and sequestering ions), then incorporated into plasma ceruloplasmin or excreted in the bile *(Brewer et al., 1992)*. In Wilson's disease, the gene encoding for a membrane-bound copper-transporting P-type ATPase (ATP7B) is defective, leading to insufficient biliary excretion of copper as well as to reduced incorporation of copper into ceruloplasmin. As a result of the positive copper balance, copper accumulates first in the liver, and later in the brain *(Cuthbert 1995)*.
The physiopathology of Wilson's disease is linked to copper toxicity, as affected organs contain higher than normal levels of copper, which is implicated in deleterious oxidation of lipids and proteins and in free radical formation *(Cuthbert 1995; Menkes 1999)*.
The natural history begins with a pre-symptomatic period of positive copper balance and copper accumulation in the liver where subclinical cirrhosis develops. The diagnosis of Wilson's disease is usually made on the basis of clinical findings such as typical neurological symptoms and/or Kayser-Fleischer corneal rings and laboratory abnormalities, for instance low serum ceruloplasmin and increased amounts of urinary copper *(Brewer 2000)*. The disease typically becomes clinically apparent in the late teens or twenties, although patients have presented as early as 3 years and as late as 60 years of age *(Anderson et al., 1998)*. Unless specific treatment is instituted, copper accumulation is progressive and ultimately fatal usually within 1-3 years of the onset of neurological symptoms, frequently as a result of hepatic failure or neurological deteriorations *(Menkes 1999)*.

The calculated prevalence of Wilson's disease was 0.6 per 10,000 EU population, on the basis of which the European Commission granted Orphan Drug status to zinc acetate dihydrate in the treatment of the condition on 31 July 2001 (EU/3/01/050).

Chelation and zinc therapy are the main treatments currently used in the treatment of Wilson's disease. Chelators such as penicillamine (authorised in the EU) and trientine (not authorised in the EU, but available for the treatment of patients who are intolerant of penicillamine) act primarily by forming complexes with copper in the blood that are excreted in the urine, and thereby very effectively reduce systemic copper to subtoxic levels. Despite excellent efficacy in halting disease progressing and even improving symptoms, many patients on chelating agents experience major adverse effects, leading to poor compliance and treatment discontinuation with recrudescence of the disease and mortality.

Zinc acetate dehydrate

Zinc acetate dihydrate (active moiety zinc cation) is not a new active substance, but a salt that has long been available and has attained compendial status.

Zinc acts primarily by decreasing the absorption of copper from the gastrointestinal tract by inducing the production of intestinal mucosal metallothionein, which binds copper so preventing its absorption *(Brewer 2000; Ferenci 1999)*.
Zinc has been used in the treatment of Wilson's disease since 1958, when Schouwink described the symptomatic improvement of two patients treated with zinc sulphate in The Netherlands (*Hoogenraad 2001*). In 1977, Schouwink's colleague, Hoogenraad started using zinc sulphate in the treatment of patients with Wilson's disease and published his promising experience of 194 patient years treatment (in 27 patients) in 1987 (*Hoogenraad 1987*). Since that time, George Brewer from the University of Michigan, USA, has undertaken the bulk of the research into the use of zinc in treating Wilson's disease, using the acetate salt because of its better tolerability compared with the sulphate (*Oelshlegel and Brewer 1977*).

Zinc aspartate and zinc orotate are authorised in Germany for the treatment of Wilson's disease. However, current information in the compendium is inadequate to allow for their use in the treatment of Wilson's disease: dosages are low and indications restricted to zinc deficiencies including dermatological indications. Zinc salts for oral administration are also available in other EU member states as nutritional supplements and magistral formulations. At the time the marketing authorisation application for Wilzin, the product was only available in the European Union on a "named-patient" or "compassionate use" basis, under the American trade name of Galzin.

The applicant has submitted documentation covering non-clinical and clinical study reports based on studies carried out by the applicant and bibliographic references. Where certain studies were lacking, adequate justifications have been given (see also non-clinical and clinical aspects). Therefore, all requirements as set out in the Annex I of Directive 2001/83/EC, as amended, were considered fulfilled.

2. Chemical, pharmaceutical and biological aspects

Composition

Wilzin is presented as hard capsules containing 25 and 50 mg of zinc as zinc acetate dihydrate. The different strengths can be distinguished by the imprinting and colour of the capsules.
The other ingredients include maize starch, magnesium stearate, hard gelatin capsule shell, titanium dioxide, blue brilliant (25 mg strength only), sunset yellow FCF (50 mg strength only) and imprinting ink.
Wilzin is supplied in HDPE bottles with a polypropylene child-resistant closure.

Active substance

Zinc acetate dihydrate is a well-known chemical entity, which is described in the European Pharmacopoeia (Ph. Eur.).
It is a white crystalline powder or leaflets and it is freely soluble in water in pH media ranging from 1.1 to 9. The chemical structure of the active substance has been briefly characterised by elemental analysis and the dihydrate structure has been confirmed by thermogravimetric analysis. The omission of any additional structural elucidation is acceptable based on the well-known nature of the active, combined with its simple structure and synthesis.

- Manufacture

Zinc acetate dihydrate is synthesised by reacting zinc oxide with glacial acetic acid, with subsequent crystallisation, separation by centrifugation, drying and milling of the crystals.
Zinc oxide and glacial acetic acid are of Ph. Eur. quality. In-process controls and corresponding specifications have been adequately defined where appropriate at each stage. No organic solvents are used during the synthesis.

- Specification

Zinc acetate dihydrate is controlled according to its European Pharmacopoeia monograph. In addition, particle size distribution is tested, but it is not expected to be a critical parameter with regards to the bioavailability of the capsules, taking into account the high water solubility of zinc acetate dihydrate.
With regards to impurities, the Ph. Eur. monograph appears to sufficiently control the impurities most likely to arise during the synthesis and storage. Related substances that may arise from glacial acetic acid have been detected in 2 batches tested but in limited quantity (levels <0.1 %), thus no additional specification has been set up for organic impurities. Concerning inorganic impurities, zinc was the only element detected in a study carried out using Inductively Coupled Plasma Analysis (detection limit ranging from 0.01% to 0.001%) in 19 elements tested including the ones limited by the Ph. Eur. monograph of zinc acetate dihydrate. Moreover, zinc acetate dihydrate is a very a stable compound, is not hygroscopic and formation of zinc oxides is very unlikely during storage.
Batch analytical data provided demonstrate conformance with the specifications. Ph. Eur. analytical methods are deemed validated.

- Stability

No formal stability studies according to ICH guidelines have been performed under long term and accelerated conditions.
The drug substance is stored in a polyethylene drum liner inside a fibre drum and, since no re-test period has been established, it will be recontrolled immediately prior to manufacture of each batch of the finished product according to Ph. Eur.

Product development and finished product

- Pharmaceutical Development

The formulation is mostly empirical. The choice of the acetate salt is partly due the observation that it induces less gastrointestinal disturbances. The two strengths have the same qualitative composition but non-proportional formula.
The function of each excipient and the rationale for its use has been satisfactorily described. For commercialisation, the colouring system and the imprinting ink of the capsule shell will be modified and the preservatives initially present will be removed. All the excipients selected are commonly used for this kind of formulation. They are of Ph. Eur. quality except some components of the capsule shell and of the imprinting ink, which are satisfactorily controlled according to different standards. All the colourants are authorised colourants in the EU and meet the specific criteria specified by the European legislation.
Compliance of the HDPE bottle and polypropylene closure selected meet the Ph. Eur. requirements for packaging materials.
The main clinical study has been performed with batches having the same or very close formula to the one proposed for registration and they were prepared using a manufacturing process essentially similar to the one proposed for production.

- Manufacture of the Product

The method of manufacture involves the following standard operations: milling, mixing, sieving and encapsulation. Adequate in-process controls have been specified.
Validation data have been provided for three consecutive commercial batches of each strength and confirm the robustness and reproducibility of the manufacturing process.

- Product Specification

The product specification includes tests for appearance, identification of zinc, identification of acetate, assay limits (release and shelf life 95.0%-105.0%), content uniformity, dissolution (NLT 75% in 30 minutes), microbial limits (Ph. Eur. – non routine test).
The specification does not include a parameter for routine control of degradation products. This is acceptable in this particular case since the manufacturing process does not involve any stressing operation and the occurrence of any degradation product is highly unlikely since zinc acetate dihydrate is a very stable and it is a non-hygroscopic compound.
Batch analysis data provided for 3-production scale batches of each strength comply with release specifications and indicate consistent and reproducible manufacture.

- Stability of the Product

3-month data for 1 full-scale development batch of each strength stored under accelerated conditions (40°C/75% RH) have been provided. Stability data have also been presented for production scale batches stored at 25-30°C/60% RH over a 2-year period.
The data provided support the proposed shelf life and storage conditions as defined in the SPC.
The applicant committed to provide additional stability data for the finished product manufactured with the new capsule shells under accelerated and normal ICH conditions.

Discussion on chemical, pharmaceutical and biological aspects

The drug substance is a well-known and a very stable chemical entity. It will be recontrolled immediately prior to manufacture of the finished product. The pharmaceutical form selected is adequate taken into account the properties and the stability of the drug substance. The excipients are commonly used for this kind of formulation and the packaging material is well documented. The manufacturing process ensures that reproducible finished product batches are produced. Based on the stability data provided so far and the good stability of zinc acetate dihydrate, the change in composition of the capsules shell for commercialisation is not expected to impact on the stability of the finished product. In addition, the applicant committed to provide additional stability data for the finished product manufactured with the new capsule shells under accelerated and normal ICH conditions.

3. Non-clinical aspects

Introduction

The non-clinical data consist entirely of reports from the scientific literature, published from 1920 to 2002, of studies using either zinc acetate or others salts (sulphate, carbonate, chloride) and also zinc oxide.

Pharmacology

- Primary pharmacodynamics (*in vitro/in vivo*)

Zn^{++}, supplied by zinc salts, acts to reduce and maintain systemic copper levels by competing for absorption on the luminal side of the intestinal epithelium, and also by inducing the synthesis of metallothionein.

Several investigators have studied the interactions between zinc and copper and/or iron in rats following administration of zinc oxide and different zinc salts including zinc acetate (in diet, or gavage, or injection):

Four studies, published in 1960, in 1967 and in 1969 precede the introduction of oral zinc therapy in Wilson's disease:

- In rats, dietary zinc oxide at dose of 0.4 % zinc (~ 1.2 g/kg), for 8 weeks, produced poor growth and anaemia. Excess dietary zinc reduced iron in the liver, and markedly reduced copper liver and copper plasma levels.

- In rats, dietary zinc (carbonate, chloride, oxide) at levels of 0.75 and 1 % (~ 0.45 and 0.6 g/kg), for 5 weeks, produced reduction in body weight gains, in liver copper and in liver iron concentrations. Excess dietary zinc also reduced blood haemoglobin levels and heart cytochrome oxidase activity. These three zinc compounds exhibited the same biological toxic effects. Addition of copper or copper + iron restored the liver copper levels and the heart cytochrome oxidase activity.
- In rats, acute zinc nitrate administration (1 mg zinc/rat) either by the intraduodenal (ID) or intraperitoneal (IP) route increased zinc plasma and liver levels. Zinc ID reduced the absorption of ^{64}Cu, and ^{64}Cu clearance from duodenal segments. Zinc IP had no effects.

- In rats, administration of excess dietary zinc (0.4 % zinc in diet) during gestation:
  - reduced serum ceruloplasmin and hemoglobin levels in dams,
  - reduced maternal and foetal tissue copper concentrations,
  - reduced cytochrome oxidase activity. The reduction of cytochrome oxidase activity and ceruloplasmin concentration is attributed to a zinc-induced copper deficiency.

  Other studies published in 1989, 1998 and 2002 including three studies with zinc acetate, support the therapeutic indication of oral zinc in Wilson's disease.

- In a rat model of Wilson's disease (= copper supplementation 100 ppm in diet, for 10 weeks), reduction of body weight gains and diarrhoea were not observed in zinc treated rats (zinc acetate, 8 weeks, p.o. gavage, at daily doses of ~ 5.7, 28.5 and 57.1 mg Zn^{++}/kg). No significant pancreatic macroscopic and histopathological changes were observed.

- In another rat model of Wilson's disease (copper 750 µg/g diet, for 11 or 8 weeks) copper loading resulted in impaired growth, enhanced liver copper, cytosol copper, and liver/intestinal metallothionein (MT). Zinc depot treatment consisted of zinc carbonate in sesame oil, administered subcutaneously (100 mg Zn^{++}/kg i.e. one depot injection) as follows:
  - Copper loading 11 weeks + zinc, 13 depot injections, for 14 weeks, mean ~ 13 mg Zn++/kg/day.
  - Copper loading 8 weeks + zinc, 9 depot injections, for 5 weeks, mean ~ 26 mg Zn++/kg/day.

  Zinc treatment significantly reduced serum SGPT, liver copper, cytosol liver copper and enhanced serum and liver zinc, intestinal copper, and, liver/intestinal MT without effecting brain MT. In rats, zinc therapy protects against copper toxicity in liver, by induction of hepatic metallothionein.

  In rats, D-penicillamine (0.5 mmol/kg/day) p.o. gavage, for five days, enhanced the urinary excretion of copper. A five-day treatment with zinc acetate (10 mg/kg/day) p.o. gavage, enhanced excretion of copper in faeces. Copper excretion (urine + faeces) is not enhanced by the co-administration of the two drugs.

- Long-Evans Cinnamon rats (LEC) were identified in Japan in 1997. This animal model has a mutation in the gene homologous to the human Wilson's disease gene, and shows most of the features of the disease, such as extensive Cu deposition in the liver and decreased serum levels of ceruloplasmin.

  LEC rats receiving 80 mg zinc acetate daily by gavage for one and two weeks showed higher levels of metallothionein in the hepatic and renal cells compared to untreated ones. Tissue Zn concentrations were significantly higher in treated rats versus controls, whereas Cu concentrations decreased in the liver and kidneys.

- Secondary pharmacodynamics

The most important metabolic effect when feeding zinc in excess is the development of anaemia (see toxicology section).
Excess dietary zinc in rats results in altered activity of numerous enzymes "ex vivo", including catalase, ferroxidase, cytochrome oxidase, xanthine oxidase and alkaline phosphatase. In most cases, excess zinc treatment has inhibitory effects on enzyme activity. Zinc inhibits intestinal alkaline phosphatase activity but enhances liver and kidney enzyme activities. Generally, these effects are observed at doses ≥ 200 mg/kg/day for periods ≥ 2 weeks (approx. 100-fold the human therapeutic dose of 2–3 mg/kg).

In rats, excess dietary zinc can also produce mild to moderate effects on the metabolism of other elements, such as inhibition of the deposition of calcium and phosphorus in bone, and the assimilation of nitrogen, phosphorus and sulphur. Zinc also causes an increase in the urinary excretion of both uric acid and creatinine.

- Safety pharmacology

The applicant performed a literature survey on the safety pharmacology of zinc salts, focusing on heart function and blood lipids. In experimental animals, Zn^{++} failed to induce cardiotoxicity (except *in vitro* with very high concentrations). Negative inotropy and chronotropy have been reported by investigators using various isolated cardiac preparations at concentrations above 5 x 10^{-6}M zinc approximately. Decreases in cardiac rate, contractile force and peak systolic pressure were observed on the isolated rat heart preparation perfused by a zinc-histidine complex.
Increases in serum cholesterol were observed in rats (2.8 or 10 mg Zn^{++}/kg/day for 2 or 7 months) but not confirmed in two additional studies. In rabbits, two studies showed a hypolipidemic effect of zinc (2.2 or 3.4 mg Zn^{++}/kg/day for 6 months i.e. similar to clinical doses).
Although data in experimental animals are limited they are over-ridden by clinical data. No unexpected adverse effects of zinc salts have been found.

- Pharmacodynamic drug interactions

No experimental animal data have been submitted. Data referring to interaction studies in animals with zinc salts are available, but they all refer to competition with other minerals or trace elements or metals. Animal findings would not be expected to contribute to the knowledge of potential interactions of zinc acetate with concomitant drugs commonly used in patients suffering from Wilson's disease.

- Summary of salient findings

The submitted publications allow concluding that Zn^{++} acts by competing for intestinal Cu^{++} absorption and by inducing the synthesis of metallothionein.
Only very high doses of zinc would be required to deleteriously alter the activity of those enzymes investigated. Theses doses are considered to be non relevant for the proposed therapeutic doses. Considering the extent of clinical experience, additional pharmacodynamic studies in animals are unlikely to extend scientific knowledge for the use of zinc in Wilson's disease. Moreover, due to clinical considerations concerning unnecessary use of animals, such studies were not repeated.

Pharmacokinetics

The available pharmacokinetic information consists of reports from the scientific literature. Only one of the studies utilizes the acetate salt form of zinc, which is proposed for clinical use.
Other studies submitted in support of the pharmacokinetic documentation used various other forms of zinc: zinc oxide or zinc salts for ADME characterisation (Absorption, Distribution, Metabolism, Excretion), after single or repeated administrations, in various species including pregnant animals.

- Absorption- Bioavailability

In a review of literature including 235 references up to 1981, evidence is provided that in humans, zinc absorption occurs through the mucosa of small intestine and against a concentration gradient Studies in rats show that zinc is primarily absorbed from the duodenum. The relative absorption of $^{65}ZnCl2$ (single oral administration) in mice, rats, dogs and humans is about 13, 23, 48 and 55 % respectively, with major excretion of zinc in the faeces
Studies in rats and chicks show that the absorption of zinc is reduced when administered with phytic acid, a soybean protein component. Some reports in rats show that calcium and phosphorus reduce the absorption of oral zinc, other reports are conflicting.

- Distribution

Distribution studies in rats show that peak tissue concentrations of ^{65}Zn are reached within 5 days after oral dosing. Distribution studies following oral long-term administrations in rats, cats and dogs, show that highest levels of zinc are attained in liver, gallbladder and bile, gastrointestinal tract, kidney, bone, bone marrow and pancreas. Average tissue zinc concentrations are analogous in cats and dogs suggesting a similar distribution.
Analysis of zinc contents in various organs and tissues showed no evidence of accumulation or tissue specific storage. In rats chronically treated (up to 53 weeks), distribution and elimination of zinc oxide and zinc salts (acetate, citrate, malate) are similar without any accumulation or tissue specific storage in all cases.

- Metabolism (*in vitro/in vivo*)

Metabolism involves incorporation of the Zn^{++} moiety into Zn-reliant enzymes
The elimination of ^{65}Zn in mice, rats, dogs and humans is triphasic with half-life values for the terminal phase of 75, 91, 94 and 154 days, respectively.
In cats, tissue levels return to normal levels (~ 2 weeks) when zinc dosing is discontinued, whereas in rats, elimination from bone and pelt is delayed.

- Excretion

Zinc oxide and zinc salts were administered in drinking water to male or female rats (n = 1 to 3/dose), for 35 to 53 weeks. Analysis of excreta (faeces, urine) from control and zinc-treated rats indicated that faecal excretion was the main route of zinc elimination. Increased doses produced only minimal increases in zinc urinary excretion.
In all species studied (mice, rats, dogs, humans), the majority of excretion occurs via the faeces, with only a small fraction excreted in the urine.

- Pregnancy

In rats, ADME of ^{65}Zn (single tracer dose) is not affected by gestation, there is a placental transfer and also a transfer of radio-zinc in milk of mother rats.

- Summary of pharmacokinetic parameters (in different species)

Only one animal pharmacokinetic study has used the intended compound, zinc acetate, and no studies were conducted to GLP. The studies are generally very old and of little qualitative value. Plasma Zn levels were not determined in these studies, interspecies comparisons and dose justifications were not possible. However, available ADME studies show concordance across salts and animal species used. Absorption is minimal in the GI tract, though modified by certain, controllable, dietary factors. Distribution studies reveal highest tissue concentrations where expected: liver, gallbladder, bile, GI tract and kidney. Metabolism involves incorporation of the Zn^{++} moiety into Zn-reliant enzymes and excretion is primarily faecal, as expected by the low level of absorption.

There are sufficient human data available to accurately deduce the clinical PK profile of the compound and clinical doses are derived from these data.

Toxicology

The applicant has provided a large number of study citations on the toxicology of zinc salts. Many of these pre-date GLP standards, but the majority are interpretable, show concordance across salts/species and, taken together, provide an adequate overall view.

- Single dose toxicity

The oral LD_{50} is about 300 mg Zn^{++}/kg for three animal species (mouse, rat, rabbit), i.e. approx. 100-150-fold the human therapeutic dose. The parenteral LD_{50} is about 10-fold lower than oral LD_{50} values, showing the poor absorption of Zn^{++} via the gastrointestinal tract. Intraperitoneal LD_{50} is about 15 mg Zn^{++}/kg in mice and rats. The intravenous LD_{50} in rats is similar.
Clinical and toxic symptoms result in depression of CNS (tremor, ataxia, dyspnoea, cyanosis) and in gastric irritation or corrosion with bleeding ulcerations and/or perforations.

- Repeat dose toxicity (with toxicokinetics)

Eight repeat-dose studies using zinc acetate, ranging from 4 weeks to 53 weeks, are available. Numerous other toxicological studies have used either zinc oxide or zinc salts. In most cases, these studies have some flaws or are limited in their design, however in general they consistently define the target organs of high dose zinc toxicity: Pancreas (histopathological changes and enzymatic inhibition); Blood (development of a specific microcytic hypochromic anaemia, reversible after supplementation with iron or copper, and more rarely bone marrow hyperplasia, lymphocyte / neutrophil inhibition); Kidney (disturbance of function with albuminuria, decreased urine volume, associated with histopathological changes); GI tract (astringent action leading to local corrosion/erosion). In addition, a general zinc toxicosis is described from animal studies (decreased bodyweight gain, vomiting and/or diarrhoea, tremor, muscular weakness, ataxia, dyspnoea and hair coat changes, associated with various metabolic changes), though this only manifests at very high doses. Indeed, copper deficiency following prolonged high-dose zinc administration is of more concern clinically.
The same target organs of toxicity are identified in rats, and also in other species, regardless of the zinc form used, i.e. zinc oxide, zinc acetate and other zinc salts.
Effects on the immune function have not been specifically studied in animals, but clinical data are available (see clinical section)
In general, the lowest doses tested and the NOEL of zinc acetate are about 18 to 95 mg Zn^{++}/kg/day (i.e. ~6-9 and ~32-48 times the human therapeutic dose) for periods ranging from 4 to 53 weeks.
Due to the clinical experience in the claimed indication, no new acute or repeated-dose toxicity studies are required.

- Toxicokinetics

Toxicokinetics of zinc salts is not well documented. No adequate studies are available to compare animal and human pharmacokinetics.
However, available ADME studies show concordance across salts and animal species used. Published data of the effects of zinc carbonate, chloride and oxide on copper and iron metabolisms in rats showed that there were no differences between the zinc salts activities; the three compounds exhibited the same biological toxic effects. Other studies conducted in rats with zinc acetate and zinc carbonate showed that the effects on copper bioavailability were obtained at quite similar doses, close to human doses (2 to 3 mg Zn^{++} /kg.), i.e. about 5.7 mg and about 13 mg Zn^{++}/kg/b.w, respectively. Finally, in toxicology, results showed no major difference between species and between zinc salts. The acute toxicity studies performed either with zinc acetate, zinc chloride or sulphate, in mice and rats (oral) lead to very close LD_{50} results and repeat-dose toxicity studies performed in rats either with zinc

acetate or other zinc salts showed that the toxicity target organs were the same regardless of the zinc salt used.

- Genotoxicity *in vitro* and *in vivo* (with toxicokinetics)

A number of genotoxic assays have been performed with zinc salts using bacteria, yeast, drosophila, plants, mammalian cells, in vitro systems and in vivo studies.
Zinc was tested in several Ames tests. Zinc acetate did not show any mutagenic activity in an assay with 5 strains of Salmonella, in the presence and absence of the S9 activation system. However, in a study where zinc was complexed with an organic ligand, the results were positive with 2 strains of Salmonella. Results from other studies with different salts were conflicting.
In the L5178Y TK+/- mouse lymphoma assay, dose-dependent positive responses were obtained in the presence and absence of the S9 metabolic activation, with a doubling of the mutation frequency occurring at ≥ 10 μg/ml. However, zinc chloride was negative using the same test in similar experimental conditions (similar range of concentrations and cytotoxicity).
In the in vitro cytogenetic CHO assay, dose-dependent positive responses of zinc acetate were obtained in the presence and absence of the S9 activation system, although the S9 reduces both the clastogenic response (≥ 34 to ≥ 45 μg/ml) and the cytotoxicity.
Zinc sulphate was negative in the in vivo mouse micronucleus test. However, a weak positivity is obtained in animals fed with a low calcium diet.

The unscheduled DNA synthesis in primary cultures of rat hepatocytes was not induced by zinc acetate.
Results of cell transformation assays (Syrian Hamster Embryo) induced by zinc chloride were equivocal: one positive and one negative. The positive assay might be explained by an epigenetic mechanism (inhibition of apoptosis).
Assays on in vitro DNA synthesis, did not show mispairing, but an inhibition of E. coli RNA polymerase or DNA polymerase at high concentration (0.5mM). Therefore, no direct interaction with nucleotides has been demonstrated in these tests unlike cadmium or manganese.

- Carcinogenicity

Published carcinogenicity studies performed with different zinc salts in mice and hamsters are inconclusive and outdated. None of them fulfil the present EU requirements.
Four carcinogenicity studies have been conducted in mice with oral zinc chloride, oleate or sulphate. Two of them are positive and two are negative. Unfortunately, study reports are only short communications or publications and are not detailed enough in order to fully assess the carcinogenic potential. On the other hand there is some agreement in the literature that oral zinc salts should not be considered as carcinogenic to man. Furthermore, the extensive genotoxic testing does not suggest that zinc has a clinically relevant genotoxic potential. Therefore, although no definitive conclusion on the carcinogenic potential can be obtained, and considering the long clinical experience of zinc salts in other indications, additional animal testing of the carcinogenic potential is deemed unnecessary for the present product with respect to the claimed indication.

- Reproductive and developmental studies

Reproduction studies performed during 2 or 3 generations showed that zinc acetate (~25 mg Zn^{++}/kg) in mice and in rats, zinc oxide (~260 mg Zn^{++}/kg) and other zinc salts at oral dose up to 85 mg Zn^{++}/kg (carbonate), have no effects on fertility and reproductive performance of male and female rats F0, F1, F2, if any. In addition, specific studies on the effects of excess dietary zinc on the chemical composition and enzymatic activities of maternal and foetal tissues have not revealed any serious adverse effects.

Oral teratology studies performed with zinc oxide in rats, with zinc sulphate in sheep, and with zinc sulphate in mice, rats, hamsters and in rabbits were always negative in terms of teratogenicity. The latest studies conducted by Food and Drug Research Laboratories in 1973 – 1974, are close to the GLP standards and to the EU regulatory requirements. Negative results were obtained with doses up to 24 and 36 mg Zn^{++}/kg in rabbits and in hamsters, respectively. For these studies, study reports were given in the dossier. It has to be stated that even the highest doses tested did not elicit any maternal toxic or fetotoxic effect and a toxic threshold level has not been determined.

Some animal experiments have indicated that gestational exposures to very high levels of dietary zinc or zinc supplements were associated with increased skeletal defects and impaired reproductive performance in mice, exencephaly in hamsters and an increased incidence of congenital malformations in rats. The high doses required to produce these effects would seem to make clinical relevance unlikely.

Only the teratology studies performed with zinc sulphate in 1973-1974 can be regarded as nearly fulfilling the present requirements for teratology testing. The poor quality of the experimental animal studies and the conflicting results do not allow a teratogenic effect to be ruled out. However, the limited clinical data suggest that incidences of miscarriage and malformation are within the normal range. In light of these results zinc acetate should be used with caution in pregnancy, but may present an advantage over other clearly teratogenic therapies.

- Other toxicity studies

Special toxicity studies have been conducted in rodents (mice, rats and rabbits), in non-rodent animals (dogs and cats) and in chicks, a non-mammal species. In all studies, the animals have been fed with the zinc compound as part of the diet or the drinking water, except for oral capsules in dogs. The objectives were to precise target organs and secondary effects of oral zinc. For these reasons the pancreatic effects and the effects on iron metabolism and zinc inducing anaemia have been investigated,

In copper overloaded rats, at doses up to 57.1 mg Zn^{++}/kg/day, zinc acetate has no effects on the pancreas. However, repeated administrations of excess oral zinc (in cats and chicks) result in the development of a pancreatic toxicity (fibrosis and reversible acinar cell vacuolation respectively) which may be copper deficiency-mediated, or due to decreases in pancreatic enzymes secondary to GI tract irritation.
A prominent preclinical feature of excess oral zinc administration in rats and dogs is the development of a microcytic hypochromic anaemia. In humans, dramatic hypochromic anaemia response to zinc supplements (oral zinc sulphate, 660 mg daily, ≥ 12 months) in patients with non-responsive celiac disease is attributed to zinc induced copper deficiency.

- Environmental risk assessment

Calculations on the environmental risk have led to the conclusion that no special precautionary or safety measures have to be taken regarding the storage of the present products, their administration to the patients and for the disposal of waste products.

Discussion on the non-clinical aspects

The submitted publications allow concluding that zinc acts by competing for intestinal Cu absorption and by inducing the synthesis of metallothionein.

Available ADME studies show concordance across salts and animal species used.

Repeat-dose toxicity consistently identifies target organs as the pancreas, haematology system, kidney and gastro-intestinal tract. The NOEL is approximately in the range of 5 to 40 times the human therapeutic dose.

Genotoxicity testing gave equivocal results. Zinc and calcium equilibrium seem to play a central role in the positive or equivocal results observed *in vitro* and *in vivo*.
In conclusion, the weight of evidence, from *in vitro* and *in vivo* tests, suggests that zinc has no clinically relevant genotoxic activity. This is expressed in the SPC, section 5.3.
Carcinogenicity testing is inconclusive due to poor available data. However, in the absence of clinically relevant genotoxic potential and considering the long clinical experience of zinc salts in other indications, additional animal testing of the carcinogenic potential is deemed unnecessary.
The available studies do not suggest that Zn^{++} affect significantly the reproductive function. However, a teratogenic effect cannot be ruled out, especially at high doses possibly by virtue of copper deficiency. Nevertheless, the use of zinc acetate during pregnancy may present an advantage over other clearly teratogenic therapies (see SPC).

Submitted bibliographical data are sufficient to describe the biochemistry of zinc salts and the potential hazards associated with their ingestion. Non-clinical data are therefore considered sufficient to fulfil requirements as set out in the Annex I of Directive 2001/83/EC, as amended.

4. Clinical aspects

Introduction

GALZIN is authorised in the USA and has been marketed by GATE Pharmaceuticals (a division of Teva Pharmaceuticals) in the USA since March 1997, as maintenance treatment in Wilson's disease for patients who have previously been treated with chelating agents. Orphan Europe SARL has proposed Teva Pharmaceuticals USA to develop GALZIN under the tradename WILZIN in the EU. At the time of the Wilzin Marketing Authorisation Application, the product was only available in the European Union on a "named-patient" or "compassionate use" basis, under the American trade name of GALZIN.

To establish the efficacy and safety of Wilzin in Wilsonian's patients, the company has submitted a bioavailability and dose ranging study of zinc acetate dihydrate in healthy volunteers (referred as NDA study because it was part of the Galzin application in the US), a dose-response study in adults and paediatric patients and a pivotal uncontrolled clinical trial (Brewer's study).
Teva Pharmaceuticals, USA who developed capsules of zinc acetate dihydrate (GALZIN), provided Dr Brewer with clinical supplies.
Those studies were supported by publications including pharmacokinetic, pharmacodynamic, clinical efficacy and safety data. Post marketing data in USA has also been submitted.

Pharmacokinetics

The applicant provided one bioavailability and dose proportionality study for zinc acetate dihydrate (25mg & 50mg) capsules in 16 healthy volunteers and a number of literature references.

Atomic absorption spectroscopy was used for the determination of zinc in human serum.

Since the mechanism of action of zinc is an effect on copper uptake at the level of the intestinal cell, pharmacokinetic evaluations based on blood levels of zinc do not provide useful information on zinc bioavailability at the site of action.

- Absorption – Bioavailability – Dose proportionality

Zinc is absorbed in the small intestine, especially in the jejunum, and probably by a carrier-mediated process. When zinc ingestion exceeds the normal dietary levels, intestinal metallothionein is induced, causing some zinc to be bound in the mucosal cells. Such zinc presumably may be lost as cells slough off.

The absorption kinetics of 25 mg and 50 mg of Wilzin has been investigated in a stepped-dose study (NDA study) in 16 healthy subjects (8 of each sex; mean age 26 years). The subjects each received a single 25 mg capsule on one day, followed by a single 50 mg capsule two days later (allowing for a

washout day) and then a 50 mg capsule three times daily for 7 doses. The pharmacokinetic results, corrected for baseline plasma levels of zinc are summarised in the table below.

Zinc dose regime	**C_{max} (μg/ml)**	**T_{max} (h)**	**AUC_{0-12h} (μg. h/ml)**	**AUC_{τ} (μg. h/ml)**
25 mg single dose	1.8	2.4	5.5	–
50 mg single dose	2.3	2.3	7.9	–
50 mg x 3/day	2.1	2.4	–	8.3

C_{max} – maximum plasma concentration; T_{max} – time of C_{max};
AUC_{0-12h} – area under the plasma concentration-time curve in the first 12 h after dosing;
$AUC\tau$ – area under the plasma concentration-time curve during the dose interval at steady state.

Based on this study, dose-linearity between 25 and 50 mg zinc is not shown, while in a published study by Henderson 1996 zinc pharmacokinetics was dose-linear up to 50 mg. Therefore clarifications were requested.

The absorption kinetics of different doses of zinc in healthy volunteers was reported in five studies (see table below). In these studies, two salts were used: zinc acetate and zinc sulphate. However, plasma zinc levels in subjects receiving either acetate or sulphate were not significantly different *(Prasad 1993)*. Thus, the data from different studies were compiled regardless of the salt administered.

Adjusted AUC ratios (25mg/50mg) in healthy volunteers

	N (age)	**Zinc salt**	**AUC 25/50**
Oelshlegel/ Brewer 1977	8	Sulphate	58%
Lee 1989	10 (18-27)	Acetate	69%*
Prasad 1993	10 (51-66)	Several	-
Henderson 1996	11	Acetate	55%
NDA Study	16	Acetate	69%

** Net Zn movement ratio with doses of 35 and 70 mg perfused over 60 min (450/650)*

Based on these studies, the AUC 25/AUC50 ratio showed a wide range of variation from 55% to 69%. This discrepancy, especially between Henderson and the NDA study, is difficult to explain. A close look at the method could not find notable differences between subjects' age (25.9±4.6 years in the NDA study versus 24.5±2.7 years for females and 26.6±3.9 years for males in Henderson study), meals and meal schedule, as well as the assay used to determine plasma zinc concentration. However, the study design was different (Henderson study was a randomised crossover study, during which a wash out period of at least 7 days between treatments was applied). It is not clear whether this difference alone can explain the discrepancy. More surprising are the differences in the level of concentration achieved in both studies in spite of the use of the same formulation (GALZIN). In the Henderson study, the concentration profile of 50 mg is quite similar to that of 25 mg in the NDA study. This could be consistent with a tendency of saturation at higher doses as demonstrated with 100 mg in Henderson study. Furthermore, the 69% ratio is similar to the ratio found with jejunal instillation (*Lee 1989* study where four doses of zinc [3.9, 35, 70, and 140 mg] were perfused in the jejunum of 10 healthy volunteers).

In conclusion, some saturation effect is already evident at a dose of 50 mg and it should be stated in the summary of product characteristics that "Zinc is absorbed in the small intestine and its absorption kinetics suggests a tendency of saturation at increasing doses."

- Bioequivalence

Up to 1 January 1986, clinical studies performed on zinc acetate dihydrate by Brewer and colleagues were with a tablet formulation manufactured at the University of Michigan Hospital Pharmacy. The dissolution profile of this formulation is unknown and so the bioavailability of zinc from it at the site of action in the intestinal wall or in terms of systemic absorption cannot be compared to that from WILZIN. However, in the analysis of clinical outcomes of patients treated by Brewer with zinc acetate dihydrate, only 37 of the 170 patients (22%) ever received the tablet formulation and all but 3 of these patients (2%) were switched to Galzin during the course of their treatment. Therefore, the vast majority of data from the analysis of clinical outcomes with zinc acetate dihydrate relates to the use of WILZIN.

Zinc acetate dihydrate and zinc sulphate show comparable systemic bioavailability.

- Distribution

In the blood, about 80% of absorbed zinc is distributed to erythrocytes; with most of the remainder being bound to albumin and other plasma proteins.

Zinc crosses the human placenta and concentrations in the cord and maternal serum are positively correlated. Zinc is excreted in human breast milk.

In Brewer's long term clinical study some patients with Wilson's disease (n=61) had hepatic zinc measurements taken over periods of up to 9 years during maintenance therapy with zinc (as the acetate) using dose regimes of 25 mg twice daily to 50 mg three times daily. In these patients an elevation of hepatic zinc levels by approximately threefold from a mean baseline level within the normal range (431 µg/g dry weight) was observed over the first 3 years of treatment, followed by a reduction to levels of 1-2 times baseline over the next 6 years. The reason for the initial marked elevation of hepatic zinc levels is unclear, but it could be to compensate for zinc deficiency caused by previous chelation therapy in some patients and/or induction of hepatic metallothionein so causing hepatic sequestration of zinc. It has also been found that mean plasma levels of zinc in a larger group of these patients (n=75) rose above the normal range (75-125 µg/dl) to levels above 200 µg/dl after 1 year of maintenance therapy and then remained at about this level during the follow up period of up to 9 years. These results indicate that systemic zinc levels are elevated above the normal range during zinc maintenance therapy for Wilson's disease, as would be expected from therapeutic dosing, but that there is no evidence that zinc accumulates during long-term administration.

- Elimination

Zinc is not metabolised. The elimination half-life of zinc in healthy subjects is in the range 0.9-1.2 hours. Elimination results primarily from faecal excretion (70-80%) with relatively little from urine and sweat (15-25%). Following oral administration urinary excretion is very low (up to 2% of the administered dose), while faecal excretion varies greatly (20%-76% of the administered dose). The latter is in the greatest part due to the passage of unabsorbed zinc but also to endogenous intestinal secretion. The contribution of bile is very small, as opposed to pancreatic secretions, which may play a significant role in the homeostasis of zinc.

- Time dependencies

The time dependency of zinc acetate dihydrate pharmacokinetics was evaluated in the NDA study. The AUC over one dosing interval following multiple dosing of the 50 mg capsules was approximately the same (less than 5% difference) as the AUC measured from dosing to return to baseline following a single 50 mg dose. The mean Cmax after multiple dosing was actually slightly lower than that observed after a single dose (after correction for baseline concentration, 2.08 vs 2.35 µg/ml, respectively). Therefore, zinc pharmacokinetics did not appear to be time-dependant.

As regards inter-individual variability: based on the dispersion parameters for the bioavailability studies, the coefficients of variation for the Henderson study were at least twice those of the NDA study; however both these results were obtained in volunteers and their significance remains unclear. No data on intra-individual variability are available.

- Special populations

Pharmacokinetic data for paediatric patients:
Zinc absorption and distribution have been extensively studied in preterm infants and adults by *Wastney (1986, 1996 & 1999)*. This team developed a compartmental model, where each pool represents zinc in different tissues or sites of body. The rate of zinc turnover between different compartments was assessed. After administration of either oral or intravenous stable isotope tracer (^{70}Zn), samples from blood, urine and faeces were analysed at different time points and data were fitted to the compartmental model. In the last paper published in 1999, nine clinically stable preterm infants (six male and three female) with gestational age of 32 ± 1 weeks were studied. The mean postnatal age was 14 ± 3 days. During the 18 ± 3 days of the trial, neonates received preterm formula containing 9.8-11.7 mg/l of zinc. One day before hospital discharge, the formula was changed to term formula with 4.9 to 5.9 mg/l of zinc. They also studied the zinc absorption and distribution in 25 adult subjects aged from 20 to 84 years. Data displayed in the table below indicate that Red Blood Cell (RDC)-Zn level was several folds higher than plasma-Zn concentration in both newborns and adults. However, the ratio of RBC-Zn/plasma-Zn in adults (14.2) was 5 times higher than the ratio in preterm infants (2.7). Despite lower fractional absorption in preterm infants (36% versus 59% in adults), zinc absorption on a body weight basis was higher in preterm infants, due to a higher zinc intake per body weight (10 fold). However, when compared to adults ingesting the same amount of zinc on a body weight basis (around 1.5 mg/kg/day), the fractional zinc absorption was much higher in preterm infants than in adults (36% versus 7%, respectively). Furthermore, in term infants aged from 2 to 7 months with mean zinc intake of 1.0 to 7.3 mg/day the mean fractional absorption varied between 29 to 54% *(Krebs 2003)*.

Zinc absorption in (pre and term) infants and adults

	Preterm infants	**Term infants**	**Adults**	
N	**9**	**45**	**25**	
Weight (kg)	1.47±0.06		69±3	
Plasma zinc (µg/dl)	98.7±5.2		86±2	162±30
RBC zinc (µg/dl)	268.1±26.2		1225±15	-
Fractional Zn absorption (%)	36±5	29-54	59±2	7±2
Zinc Absorption (µg/kg/day) **Mean** (mg/day)	464.3±52.3	 0.57-3.2	82	-
Zinc Intake (mg/kg/day) **Mean** (mg/day)	1.48	 1.0-7.3	0.145 10	≈ 1.59 110

Plasma zinc concentration in Wilson's disease paediatric patients: Individual plasma zinc concentrations for paediatric patients (aged from 12 to 19 years) were extracted from data listings in Brewer's study. Twelve patients on two dose regimes (25 or 50 mg x 3) were identified (Table below). In addition, *Brewer (2001)* reported 34 paediatric patients including the 12 patients already mentioned. All plasma zinc concentrations from paediatric patients were in the same range as those reported for the first cohort in the main Brewer study for all patients on effective doses of zinc (50x3, 50x2 & 25x3).

Plasma zinc concentrations in paediatric patients

		Plasma Zinc (µg/dl)			
Patient N°	**Dose**	**0-1 year**	**1-2 years**	**2-3 years**	**3-4 years**
42	50x3	156	223		
46	50x3		204		
55	50x3		193	135	
61	50x3		268	150	
65	50x3	189	214		
76	50x3				
95	50x3	232	88	210	176
137	50x3		218		
75	25x3	177			
117	25x3	328	228		
123	25x3	192		190	
136	25x3		206		
Mean±SD	N = 12	251±70	185±65	200±14	
Brewer's Publication (2001)					
Mean±SD	N = 34	211±52	176±49	197±54	220±44
All Patients (first cohort)					
Mean±SD	N = 86	218±74	223±55	226±65	199±63

Elderly

The systemic bioavailability of normal levels of dietary zinc is approximately 40% in young healthy subjects and about 20% in elderly subjects (*August 1989*), but increases in people in a poor nutritional state (*Spencer 1985*).

Hepatic or renal impairment

In patients with chronic renal disease, especially nephrotic syndrome and uraemia, plasma levels of endogenous zinc are decreased (*Mahajan 1989; Prasad 1979)*. The reasons for this are unclear but seem to include decreased intake and absorption in uraemia, and decreased intake and increased urinary excretion in nephrotic syndrome. It is therefore unlikely that therapeutic doses of zinc will accumulate in such patients despite the fact that it is partly excreted by the kidneys.
Cirrhosis is also associated with decreased plasma levels of endogenous zinc (*Capocaccia 1991; Prasad 1979*). The reasons for this are unclear but could include reduced intake, reduced absorption, reduced synthesis of plasma proteins that carry zinc, and porto-systemic shunting that decreases the hepatic extraction of zinc and so increases its availability for urinary excretion (*Capocaccia 1991*). It is therefore unlikely that therapeutic doses of zinc will accumulate in such patients, and such accumulation has not been noted in the studies performed on Wilson's disease patients, most of whom have some degree of cirrhosis.

Possible influence of gender, race & body weight:

Wastney 1986 & 1992 showed sex related changes of zinc metabolism with age during zinc loading. In both sexes, the response of urinary zinc increased with age; the response of plasma zinc increased with age in women only, whereas the amount of zinc absorbed increased with age in men only. The influence of race on zinc status has only been addressed in subjects with zinc deficiency. No publications addressing the influence of body weight on zinc pharmacokinetics were found.

- Interaction studies

A study of the co-administration of several foods with 25 mg zinc (as the sulphate) in healthy subjects found that many foods (including bread, hard boiled eggs, coffee and milk) impair the absorption of zinc by up to about 90% (*Oelshlegel and Brewer 1977; Nève 1992)*. The possible interaction of Wilzin with food is taken into consideration by the recommendation in the SPC that Wilzin must be taken on an empty stomach, at least one hour before or 2-3 hours after meals.

The absorption of zinc may be reduced by iron supplements, penicillamine, trientine, tetracyclines and phosphorus-containing compounds, while zinc may reduce the absorption of tetracyclines, fluoroquinolones, iron, penicillamine and trientine *(Martindale 2002)***.**

Pharmacodynamics

- Mechanism of action and Primary Pharmacology

Zinc acts primarily by decreasing the absorption of copper from the gastrointestinal tract by inducing the production of intestinal mucosal metallothionein, an intracellular protein which binds copper so preventing its absorption (Brewer 2000; Ferenci 1999).

This action has been demonstrated in 5 patients with Wilson's disease treated with GALZIN for maintenance therapy at a dose of 25-50 mg of zinc 3 times per day (Yuzbasiyan-Gurkan 1992). It was found that, 5-6 days after starting the treatment, duodenal mucosal metallothionein levels had increased by about 2-4 times and uptake of orally administered radiolabelled ^{64}copper into the blood decreased to about 1% of normal. Further increases in metallothionein levels during prolonged therapy (over 1 year) resulted in little further suppression of copper uptake. When zinc therapy was stopped, the half-life for the loss of the suppressive effect was about 11 days. This indicates that even if several doses of zinc are missed, the suppression of copper uptake will be maintained.

Another study has confirmed the marked induction of duodenal mucosal metallothionein in patients with Wilson's disease treated with oral zinc 50 mg three times daily (as the sulphate), with levels being 15 times greater compared with untreated healthy control patients ($p < 0.05$) (Sturniolo 1999). This study also found that mucosal levels of metallothionein were significantly positively correlated to duodenal mucosal levels of zinc, so supporting that the site of action of the zinc is metallothionein.

It appears that once the copper is bound to intestinal metallothionein it is excreted in the faeces as epithelial cells slough off, since faecal excretion of copper increased markedly, but after a delay of at least 1 week, in two Wilson's disease patients treated with zinc (as the acetate) 150 mg/day in divided doses (Brewer 1983).

There is also evidence in animals that zinc may also induce hepatic metallothionein, so increasing the hepatic sequestration of copper in a non-toxic form (Lee 1989b). The effect also means that hepatic levels of copper should not be used to monitor the success of zinc therapy.

Primary pharmacology data are also available through Brewer's dose-response relationship study in decoppered patients with Wilson's disease using the surrogate efficacy variable of copper balance over 10 days. This study is described below in the "Clinical efficacy section".

- Secondary pharmacology

Most secondary pharmacological effects of zinc are likely to result from copper deficiency, such as its effects on haematopoiesis, nervous system, and lipid metabolism. Others are more likely to be related to zinc itself such as its effects on the liver, pancreas and immune function. See adverse events section (laboratory findings).

Pharmacodynamic Interactions

Studies have been undertaken in patients with Wilson's disease to evaluate possible interactions between zinc acetate dihydrate and the chelating agents penicillamine and trientine (*Brewer 1993*). All patients were on maintenance therapy with 50 mg zinc 3 times a day. After a baseline 10-day copper balance study and a ^{64}copper uptake test, 5 patients also received a maintenance dose of penicillamine (250 mg 4 times daily) for 2 days, with at least one hour between dosing of each drug, and then the tests were repeated. A similar study was performed with a maintenance dose of trientine (250 mg 4 times daily) in 5 patients. Both chelators decreased the faecal loss of copper caused by zinc and yet not by enough to affect the suppression of copper uptake achieved with zinc. Furthermore, since the chelators both increased the urinary excretion of copper, there was no net effect on the copper balance achieved by zinc. In summary, the faecal loss of copper decreased while the urinary excretion of copper increased, but an adequately well controlled copper balance and ^{64}Cu absorption blockade were maintained. The mechanism of the effect of chelators on faecal loss of zinc is unknown, but since chelators are well known to bind with metal ions in the gastrointestinal tract, this could decrease the availability of zinc to induce intestinal metallothionein.

There is evidence that vitamin C may decrease the gastrointestinal absorption of copper ions by reducing them to the monovalent state (*Brewer 1993*). However, using a similar design to that described above for the chelator interaction studies, 1 g/day of vitamin C given for 6 weeks in addition to zinc maintenance therapy had no effect on faecal or urinary excretion of copper or on ^{64}copper uptake (*Brewer 1993*).

Clinical efficacy

The efficacy goal of lifelong maintenance anticopper therapy is to slowly deplete the body's excess copper and to prevent the reaccumulation of copper, thereby preventing further copper toxicity.

Brewer's study constitutes the pivotal study performed with zinc acetate dihydrate to demonstrate the efficacy of zinc treatment, as well as supportive studies from literature performed with zinc sulphate.

Overall, efficacy data is available for 255 patients, 191 symptomatic and 64 presymptomatic patients. The table below provides a summary of efficacy results on the use of zinc in treating Wilson's disease.

Source	Patient types		N	Mean duration of zinc therapy (years)	Number (%) patients with clinical and/or metabolic response	Number (%) patients who died
	Sym	Pre				
Brewer 1994,1996, 2001	126	44	170	3.2	142/157* (90)	6 (3)
Czlonkowska 1996	25	8	33	4.6	27 (82)	4 (12)
Hoogenraad 2001	19	9	28	9.1	23 (82)	5 (18)
Rossaro 1990	5		5	3.3	5 (100)	0 (0)
Case studies	16	3	19	2.6	15/18 (83)	1 (5)
Total	191	64	255		212/241 (88)	16 (6)

* evaluable for metabolic response

- Dose response study

Brewer studied the dose-response relationship with zinc acetate dihydrate as maintenance treatment for a minimum of 6 weeks in 60 well-decoppered patients with Wilson's disease (belonging to the main Brewer's study) using the surrogate efficacy variable of copper balance over 10 days. Copper balance is the daily dietary intake of copper minus its daily excretion, which should be approximately zero in a healthy individual and should be zero or slightly negative (excretion in excess of intake) in a treated patient with Wilson's disease. In this study, the threshold for positive balance (copper accumulation) was taken as 0.25 mg/day, rather than zero, because copper excretion was only measured in terms of faecal and urinary excretion, the excretion in sweat being difficult to measure. Since copper excretion in sweat is about 0.34 mg/day (*Jacob 1981*), copper balance occurs at about +0.34 mg/day when only faecal and urinary excretion are measured. Furthermore, in order to allow for a 10% error in measuring copper balance, and given that the average daily intake of copper is about 1 mg/day, reducing the threshold down to +0.25 mg/day ensures that patients who actually have a slightly positive copper balance are not considered as in balance (*Hill 1986 & 1987*).

The results of this study for all 60 patients are presented in the US Prescribing Information for GALZIN, but details of the study are only available for the first 50 patients. The copper balance results for the original and extended cohorts are presented separately in the table below. An adequate copper balance was defined as a result less than +25 mg/day.

Sufficient balance copper data (at least 10 tests) are available for five dose regimes of zinc. The recommended 50 mg x 3 regime, which has been the most extensively tested (70 tests), achieved a response rate of 91%. There is little experience with other dose regimes but the only regimes that were almost always associated with a lower response rate (≤ 80%) were those with a total daily dose of 75 mg or less and a dosing frequency of less than three times daily. The same level of control was only obtained with two other dose regimes: 50 mg x 5 and 25 mg x 3. However, these were results of short-term studies, and based on Dr Brewer's practical experience, they had the following drawbacks:

- With a higher number of daily doses, compliance might be challenging. Therefore, 50 mg x 5 is only mentioned as the maximum daily dose in the SPC.
- Conversely, a lower dose regime (25 mg x 3) has a lower safety margin, as in cases where doses are missed, the patient risks being inadequately controlled. However, provided careful monitoring is performed, this dose may be equally effective, especially in children and pregnant women who should not be exposed to excessive decoppering.

Proportions of patients with Wilson's disease who had adequate copper balance according to dose of zinc (as acetate)

Dose Regime	Daily dose (mg)	Initial cohort of 50 patients		Extended cohort of 60 patients	
		Total patients*	Patients adequately controlled (%)	Total patients*	Patients adequately controlled (%)
50 x 5	250	9	8 (89)	11	10 (91)
50 x 3	**150**	**31**	**31 (100)**	**70**	**64 (91)**
25 x 6	150	8	8 (100)	12	8 (67)
50 x 2	100	4	4 (100)	5	5 (100)
25 x 4	100	4	4 (100)	5	5 (100)
25 x 3	75	11	10 (91)	11	10 (91)
75 x 1	75	8	6 (75)	8	6 (75)
37.5 x 2	75	4	3 (75)	4	3 (75)
50 x 1	50	1	1 (100)	1	1 (100)
25 x 2	50	4	3 (75)	4	3 (75)
25 x 1	25	4	2 (50)	10	8 (80)
0	0	6	-	6	-

**Some patients received more than one dose regime or the same dose regime at widely separated intervals*

- Main study

Dr George Brewer and colleagues at Ann Arbor, Michigan, USA, have been undertaking an open-label, uncontrolled study on the use of zinc acetate dihydrate (almost entirely GALZIN/WILZIN) in the maintenance treatment of Wilson's disease since October 1980.

Methods

The study was an open, maintenance trial of zinc acetate dihydrate administered orally to patients with Wilson's disease. Each patient served as his own control. The controls, or baselines, for assessments were the patients' copper values and clinical status prior to the start of maintenance therapy.

An open design without a concurrent control group was chosen for ethical considerations. A placebo or no treatment group was considered inappropriate because of the progressive and invariably fatal nature of Wilson's disease. From an ethical standpoint it was considered unreasonable to put patients with Wilson's disease at risk of clinical deterioration while trying an experimental drug, in view of the availability of alternative effective (albeit somewhat toxic) drugs. An active treatment concurrent control group was avoided because of the known risks associated with penicillamine and trientine. Moreover, as it is generally held that penicillamine is 100% effective in the maintenance treatment of Wilson's disease, it was agreed that zinc therapy should accordingly be 100% effective to be equally efficacious. Thus a penicillamine treated patient sample was considered unnecessary to establish or reject the clinical efficacy of zinc.

The objectives of this study were to demonstrate the efficacy and safety of zinc acetate dihydrate for long-term therapy (maintenance therapy) in patients with Wilson's disease. Maintenance was defined as the period of zinc therapy when the objective was to prevent the accumulation or reaccumulation of copper and to prevent the appearance or reappearance of copper toxicity. The specific objectives were to show that zinc could:

- Maintain body copper at subtoxic levels and prevent progressive target organ damage after adequate decoppering with chelating agents, zinc or other agents in patients who were symptomatic at the start of therapy;
- Maintain body copper at subtoxic levels in patients who were asymptomatic at the star of zinc therapy;
- Maintain body copper at subtoxic levels during pregnancy without adverse effects in the mother or child.

The study participants were selected from patients with Wilson's disease who were referred to the investigator for treatment, and presymptomatic siblings who were diagnosed by the investigator from the family workup. The diagnosis of homozygous Wilson's disease was established in symptomatic patients using a combination of ceruloplasmin values, urine copper values, presence of Kayser-Fleischer rings, liver copper values and sequelae of copper accumulation. Asymptomatic patients were siblings of patients with Wilson's disease in whom one or more of the laboratory tests confirmed the diagnosis. Patients were excluded from the study if they were unable to reliably comply with the dosage or other study requirements or if they were unwilling to provide consent. Female patients of childbearing potential were initially required to use an acceptable form of contraception. However, after approximately two years of adequate control of copper in 10 female patients, these restrictions were lifted.

The maintenance period followed a period of "initial" therapy during which time the copper levels were reduced to subtoxic amounts. In general, the period of initial therapy was a minimum of two months in duration for patients with Wilson's disease who presented with elevated copper values.

The duration of zinc therapy was indefinite and lifetime treatment was anticipated unless withdrawal was clinically indicated or requested by the patient.

Patients were hospitalised at intervals of approximately twelve months, at which time neurological status was assessed, copper levels were measured and haematology, chemistry and urinalysis was performed. Additional visits were scheduled as indicated.

Urine samples were collected at 6 monthly intervals and sent through the post for copper and zinc evaluation. Measures of copper metabolism that reflected the copper status of the patient, and gave advanced warning before the risk of clinical deterioration, were used. Namely, copper balance, 24-hour urine copper, non-ceruloplasmin plasma copper, blockade of absorption of orally administered ^{64} copper, and hepatic copper were used as surrogates for the clinical control of Wilson's disease.

Results

A total of 148 patients were followed up (Data on the first 86 patients was submitted to the FDA as part of the New Drug Application in 1994 and later on a further 62 patients as an Amendment in 1996). The distribution of these patients according to their presentation and their age is shown in the table below. Paediatric patients were defined as less than 19 years old. Exactly half of the 148 patients were male. This study population is thus reasonably representative of Wilson's disease patients on maintenance therapy.

Patient population (main cohort)

	Adult patients	Paediatric patients	Total
Presymptomatic	27	4	31
Symptomatic	109 (85 N + 24 H)	8 (7 N + 1 H)	117
Total	136	12	148

N: patient with predominantly neurological /psychiatric symptoms
H: patient with predominantly hepatic symptoms/signs

17 (11%) of the 148 patients discontinued zinc maintenance therapy. In 10 cases, the patients were discharged by the investigator for being uncooperative.

In the main cohort of 148 patients, 93 (85%) symptomatic adults had already received chelators prior to starting zinc maintenance therapy. In some cases this included tetrathiomolybdate, an investigational drug which is only available in the USA and which acts by chelating with copper and protein in the gastrointestinal tract to prevent copper absorption and by chelating with copper and albumin in the blood to decrease cellular uptake of copper (Brewer 2000). It is fast acting and was favoured by Brewer's group for treating patients with a neurological/psychiatric presentation. It is usually administered for 8 weeks (Brewer 1996). Most presymptomatic adult patients (63%) were treated immediately with zinc, while the remainder (37%) received penicillamine as initial therapy before starting zinc maintenance therapy. All symptomatic paediatric patients received initial anti-copper therapy while all presymptomatic paediatric patients were started immediately on zinc.

Zinc Dose Regimens
In the main cohort, the vast majority (121/136 [89%]) of the adult patients started on a zinc dose of 50 mg three times daily, with the remainder starting on a dose of 25 mg three times daily (12 [9%]) or 50 mg twice daily (3 [2%]). In the 12 paediatric cases, 8 started on 50 mg three times daily and 4 on 25 mg three times daily. The lower doses were used primarily in younger patients and those with lower body weight.

The mean duration of treatment was 3.2 years in symptomatic adults and 3.1 years in presymptomatic adults.

Concomitant Anti-copper Therapies

In the main cohort, 52 of 109 symptomatic adults (48%) received a chelator concomitantly at some time during zinc maintenance therapy. However, in most cases (48/52), such therapy was only administered during the first year of therapy and usually at the start of maintenance therapy. This was particularly the case with tetrathiomolybdate therapy as the 8-week course overlapped with the start of zinc therapy. Of the 27 presymptomatic adults, only one patient used concomitant penicillamine over the first 2 years of treatment. In the paediatric patients three (symptomatic) were still being treated with tetrathiomolybdate at the start of zinc therapy.

Outcomes

Brewer did not define a primary efficacy variable for his study.

Brewer's overall clinical impression of the outcomes in the main cohort was that none of the first 86 patients had progression of their disease from copper toxicity during the follow-up period. Furthermore, the outcomes of the next 62 patients were considered to be comparable. However, a more objective analysis of clinical progression can be obtained by analysing the individual efficacy data for all 148 patients. Efficacy was assessed in a number of different ways:

- Effects on copper metabolism (24 hour urinary copper excretion and NCPC = Non-Ceruloplasmin Plasma Copper).
- Effect on speech and neurological function measured on integer scales.
- Effect on liver function tests (liver enzymes, bilirubin and albumin).

The measures of copper metabolism provide the best measure of control of Wilson's disease since 24 hour urinary copper excretion is invariably > 100 µg in symptomatic patients, while most symptomatic have a NCPC level >10 µg/dl. Based on Brewer's extensive experience of treating Wilson's disease patients with zinc, 24 hour urinary copper levels of ≤ 125 µg and NCPC level ≤ 25 µg/dl (the 'toxic' thresholds) indicate adequate control (*Brewer 1992*). Therefore, adequate metabolic control with zinc therapy has been defined to be when one or both of these metabolic thresholds are attained at any time during treatment. Requiring both thresholds to be attained is not desirable for several reasons. Firstly, 48% of symptomatic adults were taking concomitant chelation therapy at the initiation of zinc maintenance therapy and chelators increase urinary excretion of copper. Secondly, Brewer has found that some patients can have consistently elevated urinary copper levels over many years despite normal NCPC level and no features of clinical progression (*Brewer 1992*). Finally, the NCPC level has been found to be rather variable, perhaps because it is derived from two biological measurements, namely the total plasma copper level and the ceruloplasmin level (*Brewer 1992*). Loss of metabolic control has been defined as a persistent elevation (on two or more consecutive occasions) above the toxic threshold of a variable that was previously well controlled.

Metabolic Control in Symptomatic Adults

Of the 109 symptomatic adults in the main cohort, 7 had no data on NCPC or urinary copper and two were later found not to have Wilson's disease but other causes of liver disease. This leaves 100 cases which can be evaluated for metabolic control with zinc therapy. Of these evaluable cases, 91 patients (91%) exhibited adequate metabolic control within the first year, while another five did so during the second year and one did not do so until the fourth year. The remaining three patients were followed for 1 year or less and did not achieve adequate metabolic control during that period. Analysis of those cases in which metabolic control was not achieved until the second year shows that these patients were on concomitant tetrathiomolybdate therapy for a few weeks at the initiation of zinc treatment. The inadequate metabolic control was not associated with hepatic or neurological deterioration in four cases. In two other cases there may have been some degree of hepatic deterioration although there was not enough data to confirm this, and in one case suboptimal zinc compliance may have contributed to the effect. The three cases of inadequate metabolic control during the first year were not accompanied by hepatic or neurological deterioration. Of the patients who were well controlled during the first year, control was later lost in two cases. Analysis of these cases indicates that the loss of control was probably due to poor zinc compliance in both cases.

Metabolic Control in Presymptomatic Adults
Of the 27 presymptomatic adults in the main cohort, 4 had no data on NCPC or urinary copper, which leaves 23 cases that can be evaluated for metabolic control with zinc therapy. Out of these, 20 patients (87%) exhibited adequate metabolic control within the first year, while the 3 last patients did so during the second year. Of the patients who were well controlled during the first year, control was later lost in one case, probably due to poor zinc compliance.

Neurological Outcome in Symptomatic and Presymptomatic Patients (main cohort)
Amongst the 92 patients with a neurological presentation repeated assessments of speech were performed using a scoring system from 0 (normal) to 7 in 46 cases and neurological function using a scoring system from 0 (normal) to 38 in 39 cases. Only one patient showed clear deterioration of both scores. This patient was started on zinc from the beginning but 7 months later, she was switched on trientine for 8 months in view of persistently high urine copper and neurological deterioration; zinc was subsequently restarted. In all other patients scores were stable (sometimes with fluctuations) or showed a trend towards improvement. It should also be mentioned that no neurological symptoms appeared in the presymptomatic patients while on zinc therapy. However, this cohort includes three patients who were originally presymptomatic and developed neurological symptoms while non-compliant with penicillamine; they were later treated with zinc as part as the study and showed no further deterioration.

Liver Enzymes
Judging the significance of elevation in hepatic enzymes is difficult since these may rise transiently in Wilson's disease patients due to incidental stresses (*Brewer 1992*). However, 11 out of 148 patients in the main cohort (9 with a neurological and 2 with a hepatic presentation) showed an increase in ALT higher than twice the upper limit of normal without evidence of subsequent normalisation. They had normal pre-treatment levels except for one who had already borderline values while on tetrathiomolybdate. In six patients, these elevated values were the last data available, after a treatment period of 1 to 6.5 years, and it is therefore impossible to determine whether this raise was only transient; in the worst case (228 UI/L) an increase in AST (147 UI/L) was associated. In five patients raised values were repeatedly recorded over several years, the first occurrence being observed after 1 week (in combination with trientine) to 3 years of treatment. Overall, eight of these 11 patients were reported to be non compliant or had high urine copper levels whereas another patient was taking antidepressants which could also affect the liver function.

For effectiveness with regard to *mortality* and *treatment during pregnancy*; see clinical safety section.

- Clinical studies in special populations

Brewer has also published data on 34 paediatric patients (defined as <19 years at the time of starting therapy) treated within this study. This publication relates to the 12 patients from the main cohort, plus 22 further patients.

Paediatric patient population

	Main cohort	Published data	Total
Presymptomatic	4	13	17
Symptomatic	8 (7 N + 1 H)	9 (3 N + 6H)	17
Subtotal	12	22	34

N: patient with predominantly neurological /psychiatric symptoms
H: patient with predominantly hepatic symptoms/signs

Nineteen patients (56%) were male. The age range of patients at study entry was 3.2 to 17.7 years, of whom 11 were children (less than 12 years old) and 23 were adolescents (12 to 17 years old).

In the total paediatric cohort of 34 patients, the dosing approach was to treat those under 6 years with 25 mg zinc twice daily, those aged 6 to 15 years (if under 57 kg) with 25 mg zinc three times daily and those aged 16 years or more (or 57 kg or more) with the normal adult dose of 50 mg zinc three times daily. The actual dose regimen received by these patients is detailed below.

Dose regimen in paediatric patients

Age (years)	Dose regimen			Totals
	25mg x 2	25mg x 3	50mg x 3	
< 12	2	8	1	11
12 – 17		8	15	23
Totals	2	16	16	34

Although individual metabolic data are not available for the whole paediatric cohort of 34 patients, it is reported that only three of the whole cohort (9%) had 24 hour urinary copper levels > 125 µg and that only two amongst 33 of the cohort (6%) had NCPC > 25 µg/dl within the first year of maintenance therapy *(Brewer 2001)*. Therefore, in the worst case, 31 (91%) of the cohort had adequate metabolic control within the first year of zinc maintenance therapy. This figure is comparable with the results found in the adult groups.

A small controlled study has compared the efficacy of zinc 40-137 mg/day (as the sulphate) in 2 children (aged 8 and 12 years) with recently diagnosed Wilson's disease with that of penicillamine 500-1000 mg/day in another two children (aged 10 and 13) *(Cossack and Bouquet 1986)*. The paper does not mention how the patients were allocated to the treatments but the study does seem to have been a prospective study since a protocol was used and consent was obtained from parents before participation. Response to treatment was assessed using copper balance studies that measured the intake and urinary and faecal excretion of copper. After 4 years of zinc treatment, the copper balance was -0.09 and -0.07 mg/day while after 3 years of penicillamine treatment; it was -0.25 and -0.12 mg/day. The actual copper balance was clearly more negative than this, given that copper losses in the sweat were not measured. Therefore both treatments were effective in achieving a negative copper balance.

- Supportive studies

Data supporting the efficacy of Wilzin in treating Wilson's disease from studies and case reports were identified by literature search, in which patients were treated with zinc acetate dihydrate or sulphate, alone (monotherapy), with or without previous therapy and on a daily dose of zinc up to 150 mg/day. The results from such studies are relevant because the daily dose is as proposed and zinc sulphate is most probably bioequivalent to zinc acetate dihydrate.

Controlled study with zinc sulphate

Only one substantial controlled trial has been undertaken with zinc in Wilson's disease and this employed the sulphate salt (*Czlonkowska et al*). This study was conducted in a Neurology Unit between 1980-1992 & followed up 67 newly diagnosed presymptomatic (n=11) and symptomatic patients (n=56), the majority of whom had a neurological presentation (n=48). The age range of the patients was not stated but the mean age was approximately 29 years. Patients were allocated to receive treatment with standard doses of penicillamine (initially 1-1.5 g/day, falling to 0.75-1 g/day, n=34) or zinc 136-182 mg/day, n=33 (as the sulphate). Treatment allocation was alternate in principle but was at the discretion of the investigator. The maximum dose of zinc used in this study was slightly higher than that proposed for WILZIN (150 mg/day), but this is unlikely to result in significant additional efficacy since, as shown by the dose-response study, a dose regime of 50 mg three times daily is effective in correcting copper metabolism. Patients were permitted to switch to the other treatment during the study in the case of intolerance or inefficacy. At baseline, the two groups were similar in terms of age, duration of condition and clinical presentation, although more patients were

presymptomatic in the zinc group (8 versus 3). The effect of treatment with penicillamine and zinc is shown in the table below.

	On-treatment		Intention to treat	
Functional outcome	D-PEN (n=19)	Zn (n=29)	D-PEN (n=34)	Zn (n=33)
Asymptomatic	7 [37% (16-61)]	11 [38% (20-54)]	13 [38% (22-56)]	12 [36% (20-54)]
Improved	6 [32% (12-56)]	12 [42% (26-61)]	14 [41% (25-60)]	15 [45% (28-56)]
Not changed	1 [5% (0-26)]	3 [10% (0-27)]	1 [3% (0-15)]	-
Deteriorated	2 [10% (0-33)]	-	3 [9% (0-24)]	2[a] [6% (0-19)]
Dead	3 [15% (0-39)]	3 [10% (0-27)]	3 [9% (0-24)]	4 [13% (0-28)]

Results given as number, percentage and 95% confidence interval in parentheses

[a] *severe deterioration of neurological status was observed in one case, soon after initiation of Zn therapy*

Six symptomatic patients in each group either deteriorated clinically or died, which can be considered as lack of efficacy. All 7 patients who died (except for one) were diagnosed in a very advanced stage of the disease. Severe deterioration of neurological status was observed in only one case soon after initiation of zinc therapy. However, many more patients switched from penicillamine to zinc (10 due to side effects and 5 due to a lack of improvement) than vice versa (2 due to side effects, 1 deterioration and 1 lack of improvement) ($p < 0.003$). Efficacy is therefore better judged by considering only those patients who continued on the same therapy throughout the study (on-treatment analysis). A similar favourable clinical outcome was shown in the two groups with 79% of the patients (23/29) in the zinc group and 68% of the patients (13/19) in the penicillamine group who remained asymptomatic or improved. However, the mean duration of treatment in the penicillamine group was longer than in the zinc group (6.3 versus 3.8 years, respectively), although the authors offered no explanation. This controlled clinical study gives some evidence that the outcome of a therapy with zinc (irrespective of the salt used) in Wilson's disease is comparable to that of a therapy with penicillamine.

Uncontrolled Studies with Zinc Sulphate

Hoogenraad (1987 & 2001) has reported on the use of zinc sulphate as the sole therapy, in the treatment of 50 presymptomatic and symptomatic patients with Wilson's disease, including some children, in the Netherlands up to 1995. The dosages used were 150-1200 mg/day, in three divided doses. This is equivalent to 34-272 mg zinc, the upper end of which is significantly more than proposed for Wilzin. However, data on individual patients are available and so the demographic characteristics, previous treatments, dose and duration of zinc therapy and clinical outcomes in the 28 patients who received no more than 150 mg/day of zinc can be examined. The 28 evaluable patients comprised 15 symptomatic adults (aged 19 years or more), 5 presymptomatic adults, and 4 each of presymptomatic and symptomatic paediatric cases. Nineteen patients (68%) had previously been on penicillamine therapy, which had been stopped in some cases due to intolerance, whereas the remaining patients (32%) had not previously been treated. The overall mean duration of zinc therapy was 9.1 years (median 6.5 years). Twenty-four patients (86%) were on a zinc dose of 136 mg/day at the time when outcomes were assessed, the remainder being on lower doses. At the assessment point in 1995, a total of 23 patients (82%) had responded well to zinc therapy (stabilised, improved, asymptomatic). The other five patients all died, but only after a median of 6 years (range <1 to 12 years). This gives a mortality rate of 5/28 (18%) over a mean follow-up period of 9.1 years.

All of these patients had cirrhosis at presentation and all of them died as a consequence of liver disease (liver failure and/or after a variceal haemorrhage). In one case, the patient had changed back to penicillamine due to worsening of liver failure after an operation. No death occurred in patients with neurological symptoms. NCPC levels are available for nine of the above patients 10 years before the latest assessment of clinical outcomes (i.e. 1985), after a mean of 8 years treatment (median 3 years) (Hoogenraad 1987). In all cases NCPC was normal (≤ 10 mg/dl).

In another uncontrolled study, five patients aged 14-29 years (two under 19 years old) with symptomatic Wilson's disease received 50 mg zinc (as the sulphate) three times daily following previous penicillamine therapy, which had been stopped in three cases because of intolerance *(Rossaro 1990)*. After a mean treatment duration with zinc of 3.5 years (range 1.5 to 7 years), all patients were either clinically improved (4 cases) or clinically stable (1 case), i.e. a response rate of 100%. In all cases 24 h urinary copper excretions normalised.

Case reports

Case reports have been identified for 17 patients who have been treated with zinc sulphate (16 cases) or zinc acetate dihydrate (1 case) at daily zinc doses within, or less than, that proposed for Wilzin.

Amongst the 10 adult cases, 8 of whom had previously been treated with penicillamine, clinical and copper metabolic changes were reported in all but one case. In six of the nine evaluable cases, patients either improved or were stable on zinc therapy. Two other patients deteriorated soon after zinc initiation: acute hepatitis in a presymptomatic patient (with only raised transaminases) who recovered on penicillamine (Castilla-Higuero 2000) and hepatic failure with fatal neurological deterioration in an asymptomatic patient who had been treated with repeated courses of penicillamine (10 years in total) but had stopped any therapy for the preceding 3 years (Lang 1993). A third patient, who had been on a low daily dose of 50 mg of zinc for 3 years, lost adequate copper control and developed jaundice due to acute haemolysis (Shimon 1993).

Amongst the seven paediatric cases (age range at start of zinc therapy 4-16 years), 5 of whom had previously been treated with chelators, clinical and copper metabolic changes were reported in all cases. All patients either improved or were stable on zinc therapy. The report of an increased hepatic copper level in one case (Walshe 1984) is not indicative of a metabolic deterioration, since zinc is thought to have this effect by inducing hepatic metallothionein.

Therefore, case studies from the literature confirm that most patients (81%) exhibit clinical stabilisation or improvement, so long as an adequate dose is used.

- Discussion on clinical efficacy

The efficacy goal of lifelong maintenance anticopper therapy is to slowly deplete the body's excess copper and to prevent the reaccumulation of copper, thereby preventing further copper toxicity. Evidence for the efficacy of zinc in the proposed dose regime as maintenance therapy in Wilson's disease is provided by the data accumulated for more than 40 years of use, first in the Netherlands and later in the US and other European countries. Overall, efficacy data is available for 255 patients, 191 symptomatic and 64 presymptomatic patients.

Although the section on clinical efficacy deviates from usual standards (e.g. lack of well controlled dose-finding studies, pivotal study non-controlled, only descriptive statistics where indicated), there is clear evidence that zinc acetate dihydrate is effective in patients with Wilson's disease.

Brewer's study, which constitutes the pivotal study, as well as supportive studies from literature, demonstrates the efficacy of zinc treatment based on:

- Reduction of urine copper which reflects the reduction of the body load of copper; decrease in non ceruloplasmin (free) plasma copper, which is the potentially toxic copper in the blood;
- Stabilisation or improvement of the clinical symptoms and liver function tests in most symptomatic cases;
- Prevention of the occurrence of clinical signs/symptoms in presymptomatic patients;
- Data survival as compared to the natural history of the disease

These data establish efficacy of Wilzin in symptomatic patients who have been decoppered by chelating treatment and whose disease has stabilised, and, in presymptomatic patients without previous chelating treatment.

When switching a patient on chelating treatment to Wilzin for maintenance therapy, the chelating treatment should be maintained and co-administered for 2 to 3 weeks since this is the time it takes for the zinc treatment to induce maximum metallothionein induction and full blockade of copper absorption.

No data support the use of combination therapy in the initial treatment phase of symptomatic Wilson's disease patients exists (except for very severe cases).
In principle, zinc acetate dihydrate is not recommended for the initial therapy of symptomatic patients because of its slow onset of action; symptomatic patients must be initially treated with a chelating agent and once copper levels are below toxic thresholds and patients are clinically stable, maintenance treatment with Wilzin can be considered.
Nevertheless, while awaiting zinc induced duodenal metallothionein production and consequential effective inhibition of copper absorption, zinc acetate dihydrate could be administered initially in symptomatic patients in combination with a chelating agent.

Considering the fact that patients with Wilson's disease are generally treated at specialist centres by physicians with experience and expertise in this area, then it was agreed that the more general term "treatment of Wilson's disease" was the most appropriate indication.

Adequate metabolic control was also obtained in the paediatric cohort. Data are very limited in children under 6 years but since the disease is fully penetrant, prophylactic treatment should be considered as early as possible. The recommended dosage is as follows:

The recommended posologies in adults (50 mg x 3 to x 5 daily), children or adolescents from 1-6 years (25 mg x 2 daily), from 6-16 years if bodyweight under 57 kg (25 mg x 3 daily) from 16 years or bodyweight above 57 kg (50 mg x 3 daily) and in pregnant women are in line with the posologies used in the pivotal study. In all cases, dosage should be adjusted according to therapeutic monitoring. The aim of the treatment is to maintain the plasma free copper below 250 microgram/l (normal: 100-150 microgram/l) and the urinary copper excretion below 125 microgram/24 h (normal: < 50 microgram/24 h).

Applicant's own generated data, together with the published literature, are sufficient to fulfil normal dossier requirements and the application can be considered a full 'mixed' application in accordance with art. 8 (3) and Annex I part II.7 of Directive 2001/83/EC, as amended.

Clinical safety

- Patient exposure

The extent of exposure to zinc (as the acetate or sulphate) in clinical studies and case studies in Wilson's disease at daily doses similar to that proposed in the SPC are summarised in the tables below.

Zinc exposure in Brewer's uncontrolled study with zinc acetate dihydrate

Patient group	Daily dose of zinc (mg)	N	Mean duration of zinc therapy (years)	Patient-years of zinc therapy
Symptomatic adults	75-150	109	3.2	345
Presymptomatic adults	75-150	27	3.1	83
Paediatric patients	50-150	34	3.6	121
All patients	50-150	170	3.2	549

Zinc exposure in adults in other studies and case reports using zinc sulphate[1]

Source	Daily dose of zinc (mg)	N	Mean duration of zinc therapy (years)	Patient-years of zinc therapy
Czlonskowska 1996	136-182	33	4.6	152
Hoogenraad 2001	68-136	20	10.2	204
Rossaro 1990	150	3	2.6	8
Case studies	45-150	10	1.6	16
All patients	45-182	66	5.8	380

[1]Except that zinc acetate dihydrate was used in one case report

Zinc exposure in paediatric patients (< 19 years old) in other studies and case reports using zinc sulphate

Source	Daily dose of zinc (mg)	N	Mean duration of zinc therapy (years)	Patient-years of zinc therapy
Hoogenraad 2001	34-136	8	6.5	52
Rossaro 1990	150	2	4.4	9
Case studies	40-150	9	3.8	34
All patients	34-150	19	5.0	95

In Brewer's study on zinc acetate dihydrate a total of 170 patients were treated for 549 patient-years. In addition, a further 66 adults were treated with zinc (as the sulphate in 65 cases) for a total of 380 patient-years, and a further 19 paediatric cases were treated with zinc (as the sulphate) for a total of 95 patient-years. In all of these cases, the daily dose of zinc used was within the range proposed in the summary of product characteristics (SPC).

Therefore, a total of 255 patients with Wilson's disease have been treated with zinc salts at daily doses very similar to those proposed in the SPC for a total of 1024 patients-years. This includes a significant exposure in paediatric cases of 53 patients for a total of 216 patient-years.

It is estimated that since GALZIN was marketed in the USA, a further 1045 patient-years of exposure has occurred.

- Adverse events

Adverse events occurring during the use of zinc salts in maintenance therapy for Wilson's disease have only been systematically reported in Brewer's study with zinc acetate dihydrate in 170 patients.

Adverse events were reported in 27 of these patients, the most common being gastric irritation, which was reported in 15 cases (8.8%). This effect was considered to be treatment related, but was usually confined to the first morning dose and was minimised by a change in the timing of the first daily dose to mid-morning or co-administration with food. However, of the 11 patients who reported gastric irritation within Brewer's main cohort of 148 patients, 10 had started on a zinc dose of 50 mg three times daily out of a total of 129 patients on this dose, i.e. 7.8%.

In contrast, the other patient had started on a zinc dose of 25 mg three times daily, out of a total of 16 patients in this dose, i.e. 6.3%. Although, these data are consistent with gastric irritation not being dose related, the possibility of a dose relationship cannot be excluded, given that so few patients were exposed to the lower dose of zinc.

The next most common adverse events reported in this study were weight loss (4) and pneumonia (3). However, in the case of weight loss, two sibling patients also suffered from multiple sclerosis and in another case the patient was severely disabled with Wilson's disease. This leaves only one case of weight loss that cannot be otherwise explained. Among the three cases of pneumonia, two occurred during institutionalisation and one was an aspiration pneumonia due to dysphagia.

The only other adverse event reported in more than one patient was fatal accidents. One patient died in a road accident and another died in a drowning accident. All other adverse events were reported only once.

Therefore, gastric irritation is the only adverse event for which there is good evidence of a causal link to zinc.

Adverse Events in Conditions other than Wilson's disease:
In a study on elderly patients (mean age 71-72 years) with venous leg ulcers, the tolerability profile of zinc at daily doses of 150 mg (as the sulphate) in 27 subjects was compared with that in 29 patients treated with placebo using alternate allocation (*Tschumi and Floersheim 1981*). The two groups were comparable demographically and in terms of current illnesses at baseline. Over 3 months, no adverse effects were attributed to zinc therapy and haematological, renal and hepatic laboratory variables were not clinically significantly different between the groups**.**

- Serious adverse event/deaths/other significant events

In the pivotal study, the rate of death was 6 out of 170 patients (3.5%) treated with zinc and 5 out of 168 patients (3%) who had Wilson's disease. The causes of death were related to intercurrent or residual Wilson's diseases. No adverse events were reported as 'serious' in this study. However, other than the cases of death discussed above, one case each of variceal bleeding secondary to underlying cirrhosis and HIV diagnosis would probably be considered serious but are unlikely to be related to zinc therapy.

In the comparative study of zinc sulphate versus penicillamine in 67 patients with Wilson's disease, the death rate was the same in both groups (4 versus 3 cases), and there is indication that these deaths were the result of disease progression in patients diagnosed in a very advanced stage *(Czlonkowska 1996)*. However, one patient developed severe anaemia caused by bone marrow depression after 2 years of zinc therapy; she also had a very low level of total serum copper at that time. She recovered after symptomatic treatment and was switched to penicillamine.

In the cohort of 28 patients followed by Hoogenraad, five patients with liver cirrhosis at presentation died from complication of their liver disease (Hoogenraad 2001).

Therefore, there is no evidence from clinical studies with zinc acetate dihydrate or sulphate in patients with Wilson's disease that zinc caused any serious adverse event apart from haematological complications secondary to excessive decoppering.

- Laboratory findings

Haematological Effects

In the study by Brewer in 170 patients with Wilson's disease, no case of anaemia or leucopenia was reported and no trend in mean levels of haemoglobin, white cells or platelets was found during the study. A few isolated low values of haemoglobin in females have been attributed to heavy menstrual bleeding and low values of WBC and platelets counts have been attributed to hypersplenism.

Only two cases of anaemia/leucopenia were reported in the literature: one of which has already been described *(Czlonkowska 1996)*; and the other is a boy who started zinc at the age of 14 years (45 mg three times daily as the sulphate) and developed microcytic anaemia and neutropenia 12 months later, in association with copper deficiency (low serum and urine copper); he recovered rapidly following transient reduction of the daily dose of zinc *(Alexiou 1985)*. This emphasises the need for careful monitoring of the copper metabolism during long-term zinc therapy.

With zinc supplementation becoming popular among the general public it was recognised that ingestion of zinc, even at daily doses less than 150 mg, can cause bone marrow depression with leucopenia (especially neutropenia) and anaemia. This has been attributed to copper deficiency rather than zinc toxicity and low levels of serum copper and ceruloplasmin were detected in all these cases. Full recovery was obtained after discontinuation of zinc and sometimes copper supplementation *(Fiske 1994)*.

Anaemia may be micro-, normo- or macrocytic and is often associated with leucopenia. Bone marrow examination usually reveals hypocellularity, cytoplasmic vacuolisation of both myeloid and erythroid precursors, and characteristic "ringed sideroblasts" (i.e. developing red blood cells containing iron-engorged paranuclear mitochondria). A number of mechanisms have been postulated to explain anaemia and neutropenia. Low levels of ceruloplasmin and cytochrome oxidase, a copper-dependent enzyme involved in heme synthesis, may impair iron mobilisation, mitochondrial transport and utilisation *(Porea 2000)*. An interference with the gastrointestinal absorption of iron is less likely because iron deficiency is usually not reported.

Cases of sideroblastic anaemia have also been reported with penicillamine and trientine; copper deficiency is one of the likely mechanisms. However, other mechanisms may be involved explaining why haematological toxicity is more frequent, especially with penicillamine, such as iron deficiency, pyridoxine antagonism and hypersensitivity; fatal aplastic anaemia, agranulocytosis and thrombocytpenia have been reported with penicillamine *(Walshe 1982, US Prescribing Information for Trientine and Penicillamine)*.

Clearly, patients with Wilson's disease having a systemic excess of copper are less at risk of copper deficiency, which explains why anaemia is so rare with zinc therapy.

It has also been reported that large doses of zinc (300 mg/day) over 6 weeks can impair lymphocyte and neutrophil function in healthy men *(Chandra 1984)*. There is, therefore, a theoretical concern that long-term treatment with zinc could cause suppression of the immune system and increased susceptibility to infection. However, lymphocyte function was not found to be impaired in 11 Wilson's disease patients from Brewer's study who were treated with zinc 150 mg/day for at least 5 years, compared with healthy control subjects (Brewer 1997).

Pancreatic Effects

In an early group of patients from Brewer's study, therapeutic doses of zinc acetate dihydrate were found to cause elevations of serum levels of the pancreatic enzymes, lipase, amylase, alkaline phosphatase to slightly above the normal range after a few weeks of treatment, which returned to the high normal range after about 1 year. Although suggesting pancreatitis, these changes were not accompanied by any clinical symptoms of pancreatitis or cholestasis and they are thought to be due to a benign induction of the enzymes in the pancreas and liver *(Yuzbasiyan-Gurkan 1989)*. The data from all 170 patients for whom data was available confirm that zinc therapy is associated with a rise in

levels of lipase, amylase and alkaline phosphatase to the upper limit of the normal range over the first year. Levels then appear to decrease slightly over subsequent years.

Furthermore, although the amylase and lipase levels of two patients with Wilson's disease who were given very high, supratherapeutic doses of zinc (200 mg four times daily) for 10 days, increased markedly, levels had returned to normal on follow up 6 months later, and no symptoms of pancreatitis were reported. In addition, when rats were administered up to 25 times the therapeutic dose of zinc acetate on a weight-for-weight basis for 2 months, no lesions of the pancreas were found. This effect of zinc therefore seems to be due to benign induction of the enzymes, given that human alkaline phosphatase is a known zinc metalloenzyme and, in bacteria at least, so are amylase and phospholipase *(Yuzbasiyan-Gurkan 1989)*.

Liver Enzymes

Judging the significance of elevation in hepatic enzymes is difficult since these may rise transiently in Wilson's disease patients due to incidental stresses *(Brewer 1992)*. However, 11 out of 148 patients in the main cohort (9 with a neurological and 2 with a hepatic presentation) showed an increase in ALT higher than twice the upper limit of normal without evidence of subsequent normalisation. They had normal pre-treatment levels except for one who had already borderline values while on tetrathiomolybdate. In six patients, these elevated values were the last data available, after a treatment period of 1 to 6.5 years, and it is therefore impossible to determine whether this raise was only transient; in the worst case (228 UI/L) an increase in AST (147 UI/L) was associated. In five patients, raised values were repeatedly recorded over several years, the first occurrence being observed after 1 week (in combination with trientine) to 3 years of treatment. Overall, eight of these 11 patients were reported to be non compliant or had high urine copper levels, whereas another patient was taking antidepressants which could also affect liver function.

Blood Lipid Effects

There have been some reports in literature that treatment with zinc at doses of 50-300 mg/day causes a reduction in high density lipoprotein (HDL) cholesterol in males but not females *(Hooper 1980; Freedland-Graves 1982; Chandra 1984; Black 1988)*. This observation raises a potential concern that long-term treatment with zinc could be atherogenic.

This issue has been investigated in detail in a subset of 11 female and 13 male Wilson's disease patients from Brewer's study *(Brewer 1991)*. A statistically significant reduction in total cholesterol (by about 10%) and in HDL cholesterol (by about 20%) was observed over a mean treatment period of about 2 years, but only in males. There were no statistically significant changes in either variable in females, or in triglyceride, low-density lipoprotein cholesterol levels or the ratio of total cholesterol to HDL-cholesterol in either sex. Since the latter ratio is an overall measure of cardiovascular risk, taking into account deleterious effects of a low HDL-cholesterol and the beneficial effect of a low total cholesterol, the overall cardiovascular risk in both sexes was not altered by zinc therapy. This was later confirmed in the whole adult cohort of Brewer's study.

However, in the paediatric cohort from Brewer's study, the mean HDL/total cholesterol ratio decreased by about 10% from 0.295 to 0.267 ($p=0.004$) during the first year of zinc maintenance therapy *(Brewer 2001)*. Although these effects are modest and probably without cardiovascular consequence at this age, they may indicate some degree of copper deficiency due to over treatment, as these results are similar to those found in animals or humans depleted of copper *(Klevay 2001)*.

Renal function

There were no notable changes in mean levels of, or clinically significant changes in, serum creatinine, blood urea nitrogen, and urinary protein levels in Brewer's study.

- Safety in special populations

Use in Pregnancy and Lactation

The available data on the use of zinc salts during pregnancy in patients with Wilson's disease comprise 42 pregnancies in 25 patients from Brewer's study with zinc acetate dihydrate *(Brewer 2000)* and Hoogenraad's study with zinc sulphate *(Hoogenraad 2001)*. No other cases of the use of zinc therapy for Wilson's disease during pregnancy have been found in the literature.

In Brewer's group of 19 patients (7 presymptomatic, 8 with neurological, 3 with hepatic, 1 with unknown presentation) 30 pregnancies resulted in four miscarriages and 26 live births. Zinc therapy was used throughout gestation in all except two cases, in which it was started in the second month. At the end of gestation, zinc was administered at the daily dose of 25-75 mg in most cases (62%); in 31% of the cases the daily dose was maintained at 150 mg and in 2 cases (8%) the dose had to be increased to 200 mg. Two infants from these 26 pregnancies had a congenital abnormality: microcephaly, which resulted in death one hour after birth and a correctable heart defect which required surgery at 6 months of age. It is of interest to note that a low serum copper concentration in pregnant women during midgestation is a risk factor for anencephaly *(Buamah 1984)* and that both mothers had relatively low levels of copper in urine and blood.

In Hoogenraad's group of 6 patients with 12 pregnancies, all patients were treated with 136-205 mg of zinc daily in three divided doses. One pregnancy ended with a miscarriage, 10 with normal live births and in one case the foetus died at 28 weeks gestation when the patient, who had portal hypertension, died of variceal haemorrhage. In this last case, no assessment of the effect of zinc therapy was made.

Pooling data from these two studies: from a total of 42 pregnancies, 36 resulted in live births, 5 in miscarriages (11.9%) and one was prematurely ended because of maternal death. Of 36 live births there were two cases with congenital abnormalities (5.6%). The miscarriage rate is similar to that experienced in the general population of 10-15%. The rate of major congenital abnormalities may be somewhat higher than that found in the general population of about 2-4%, although this cannot be confirmed because of the very small sample size.

Zinc is known to appear in breast milk in humans (Byczkowski 1994; Krachler 1999) and so zinc-induced copper deficiency could develop in breast-fed babies.

Hepatic impairment:

Patients with cirrhosis (as in Wilson's disease) tend to have reduced plasma levels of zinc when given therapeutically. It is therefore unlikely that therapeutic doses of zinc will accumulate in such patients, and such accumulation has not been noted in the studies performed on Wilson's disease patients, most of whom have some degree of cirrhosis.
Two patients of a series of 16 died from hepatic decompensation and advanced portal hypertension after being changed from penicillamine to zinc therapy. Therefore caution should be exercised when switching patients with portal hypertension from a chelating agent to Wilzin, when such patients are doing well and the treatment is tolerated.

- Safety related to drug-drug interactions and other interactions

Possible interactions of zinc acetate dihydrate with other medicines do not seem to be relevant with respect to safety issues.

- Discontinuation due to adverse events

Only one patient is known to have discontinued therapy due to an adverse event in Brewers study of zinc acetate dihydrate as maintenance therapy in 170 patients with Wilson's disease. This was due to nausea in a patient with gastric irritation due to the zinc therapy. This is a very low rate of discontinuation due to intolerance (<1%).

In the larger of the controlled studies in which zinc sulphate (at zinc doses similar to those proposed in the SPC) was compared with penicillamine (at standard doses) in 67 patients with Wilson's disease, adverse events causing treatment discontinuation were also systematically reported (*Czlonkowska 1996*). Overall, the discontinuation rate due to adverse events was clearly greater with penicillamine than zinc sulphate (2/33 [6%]). The causes of these discontinuations were and abdominal pain (1 case) and anaemia (1 case) with zinc sulphate.

- Post marketing experience

GALZIN has been marketed by Teva Pharmaceuticals in the USA since March 1997 as maintenance treatment in Wilson's disease for patients who have previously been treated with chelating agents.

Based on the sales up to the end of 2001 and assuming that 50 mg and 25 mg capsules are used in the recommended dosage regimes, an exposure to about 1045 patient-years of treatment has been estimated. During this period, Teva received no reports of serious adverse events and reports of non-serious adverse events in only four patients, as follows:

- Hallucinations likely due the progression of Wilson's disease.
- Dyspepsia and severe lower abdominal cramps with nausea and vomiting after increased dose to 50 mg t.i.d.
- Burning in throat followed by cough after taking GALZIN for an unclear indication (probably part of a wide range of nutritional supplements used by the patient).
- Anaemia and two large bleeding gastric ulcers.

No ADR report was received in 2002 and there was one report of nausea and vomiting in 2003.

- Discussion on clinical safety

Available studies and case reports detail a total of 255 patients who have been treated with zinc acetate dihydrate or sulphate at daily doses very similar to those proposed in the SPC for a total of 1024 patients-years. This includes a significant exposure in paediatric cases of 53 patients for a total of 216 patient-years. In addition, it is estimated that since GALZIN was marketed in the USA a further 1045 patient-years of exposure has occurred. For an orphan indication, this represents a substantial body of experience.

Despite this substantial exposure, the only adverse event that has been consistently (less than 10%) associated with the use of zinc salts is gastric intolerance and this rarely leads to treatment discontinuation. The four manifestations of digestive intolerance, which have been reported post-marketing and may be related to zinc, represent a very low reporting rate of adverse events in the context of over 1000 patient-years of treatment.

Overtreatment carries the risk of copper deficiency, which is especially harmful for children and pregnant women.

Elevations of serum alkaline phosphatase, amylase and lipase may occur after a few weeks of treatment, with levels usually returning to high normal within the first one or two years of treatment.

In addition to copper monitoring, laboratory follow-up including haematological surveillance and lipoproteins determination should be performed to detect early manifestations of copper deficiency, such as anaemia and/or leukopenia, and decrease in HDL cholesterol and HDL/total cholesterol ratio.

Non-clinical reproduction toxicology studies performed with different zinc salts showed no evidence of embryotoxicity, foetotoxicity or teratogenicity. Data on a limited number of exposed pregnancies in patients with Wilson's disease give no indication of harmful effects of zinc on embryo/foetus and mother. Wilzin should only be used during pregnancy when clearly necessary. It is extremely important that pregnant Wilson's disease patients continue their therapy during pregnancy. Which treatment should be used, zinc or chelating agent should be decided by the physician. Dose adjustments to guarantee that the foetus will not become copper deficient must be done and close monitoring of the patient is mandatory.

Breast-feeding should be avoided during Wilzin therapy because zinc-induced copper deficiency in the breast-fed baby may occur.

Applicant's own generated data, together with the published literature, are sufficient to fulfil requirements as set out in the Annex I of Directive 2001/83/EC, as amended..

5. Overall conclusions, benefit/risk assessment and recommendation

Quality

The quality of this product is considered to be acceptable. At the time of the CHMP opinion there were some outstanding quality issues which were not deemed to have any impact on the benefit/risk profile. Thus, the applicant committed to provide the necessary information as follow-up measures within an agreed timeframe, and to submit variations if required following the evaluation of this additional information.

Non-clinical pharmacology and toxicology

Preclinical studies have been conducted with zinc acetate and with other zinc salts. Pharmacological and toxicological data available showed large similarities between zinc salts and among animal species. The weight of evidence, from *in vitro* and *in vivo* tests, suggests that zinc has no clinically relevant genotoxic activity. Reproduction toxicology studies performed with different zinc salts showed no clinically relevant evidence of teratogenicity. No conventional carcinogenicity study has been conducted with zinc acetate dihydrate, but considering the absence of relevant genotoxic effects and the long-term clinical experience, such studies are not considered necessary.

Efficacy

Wilson's disease (hepatolenticular degeneration) is an autosomal recessive metabolic defect in hepatic excretion of copper in the bile.

The active moiety in zinc acetate dihydrate is zinc cation, which blocks the intestinal absorption of copper from the diet and the reabsorption of endogenously secreted copper. Zinc induces the production of metallothionein in the enterocyte, a protein that binds copper thereby preventing its transfer into the blood. The bound copper is then eliminated in the stool following desquamation of the intestinal cells.

The efficacy goal of lifelong maintenance anticopper therapy is to slowly deplete the body's excess copper and to prevent the reaccumulation of copper, thereby preventing further copper toxicity.

Wilzin in three administrations was shown to be effective in significantly reducing copper absorption and inducing a negative copper balance.

Brewer's study as well as supportive studies from literature demonstrates the efficacy of zinc treatment by ensuring copper balance, improving clinical symptoms and liver function tests in most symptomatic cases, and, preventing the occurrence of clinical signs/symptoms in presymptomatic patients. Adequate metabolic control was also obtained in the paediatric cohort.

Dosage should be adjusted according to therapeutic monitoring. The aim of the treatment is to maintain the plasma free copper below 250 microgram/l and the urinary copper excretion below 125 microgram/24 h. Wilzin treatment should be initiated under the supervision of a physician experienced in the treatment of Wilson's disease.

Safety

The only adverse event that has been consistently (less than 10%) associated with the use of zinc salts is gastric intolerance and this rarely leads to treatment discontinuation. Laboratory monitoring is

necessary to prevent overtreatment with risk of copper deficiency, which is especially harmful for children and pregnant women.

Benefit/risk assessment

Given the invariably fatal nature of the disease, the efficacy and safety profiles of zinc as described, and the relative toxicity of currently available treatments for Wilson's disease, the overall risk benefit assessment of Wilzin is positive.

Recommendation

Based on the CHMP review of data on quality, safety and efficacy, the CHMP considered by consensus that the benefit/risk ratio of Wilzin in the treatment of Wilson's disease was favourable and therefore recommended the granting of the marketing authorisation.

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