1. SCIENTIFIC DISCUSSION

1.1 Introduction

Problem statement

Severe myoclonic epilepsy in infancy (SMEI, Dravet's Syndrome) is a recently defined condition first described in 1978 by Dr C. Dravet (Dravet, 1978; Dravet et al, 1982, 1985). The condition has recently been designated as Dravet's syndrome in the last Classification of Epilepsy syndromes by the International League against Epilepsy (ILAE). It is characterised by family history of epilepsy or febrile convulsions, with generalised or unilateral seizures beginning during first year of life, with secondary development of myoclonic jerks and partial seizures. Psychomotor development is retarded from the second year of life onwards, including development of ataxia, pyramidal signs and interictal myoclonus. Experience with this form of epilepsy shows it to be very resistant to most forms of currently available treatment.
The prognosis of SMEI is therefore very unfavourable for cognitive development and epilepsy. The average development quotient of most affected subjects varies between 20-40 after ~5 years of age (limited data). The mechanisms of the protracted impact on cognitive function in SMEI are still unknown. Several factors are suspected which could each partly contribute to the secondary appearance of mental retardation in SMEI children:
a) one of these factors could be the genetic mutation identified in about one third of the patients in the SCN1A gene, that codes for a protein of sodium channel (Nabbout et al, 2003). Nevertheless, the majority of patients with SMEI do not exhibit any mutation in SCN1A and on the other hand, some healthy relatives may carry the mutation.
b) another factor of cognitive aggravation is likely the high risk of prolonged seizures resulting in status epilepticus (Dravet et al, 1992): in the Marseille's neuropsychological series, the age of onset of deterioration and its magnitude was related to the frequency and the duration of seizures (Casse-Perrot et al, 2001). There is therefore a cognitive challenge to try to reduce seizure frequency in SMEI patients.
Among childhood epilepsies, severe myoclonic epilepsy in infants (SMEI) is one of the most deleterious epilepsy syndromes reported in the syndromic classification of the International League Against Epilepsy. The stereotyped clinical characteristics and the absence of any cerebral lesion make SMEI a nosologically and aetiologically homogeneous syndrome. Seizures appear during the first year of life and never come under complete control with conventional antiepileptic drugs. All children develop mental retardation in the second year of life, although development is normal before that time. These patients may be worsened by vigabatrin and lamotrigine.

About the product

Stiripentol was designated (5 December 2001) as an orphan drug for its use in severe myoclonic epilepsy in infants (SMEI).
Stiripentol belongs to a family of α-ethylene alcohols with activity in the central nervous system.
The chemical formula for Stiripentol is 4,4-dimethyl-1-[3,4-(methylendioxy)-phenyl]-1penten-3-ol.
It is a chiral molecule centralised around one asymmetric carbon atom C3. Both enantiomers are active, the R (+) enantiomer being ~2.5 times more active than the S (-) enantiomer based on animal studies. The drug substance is an equimolar racemate, which has been used in all studies in man.
The anticonvulsant activity of Stiripentol is not known, but it is supposed that it could be due partly by direct anticonvulsant activity related to effects on GABA and also by potentiation of the efficacy of some other antiepileptics as the result of pharmacokinetic or pharmacodynamic interactions. In particular: a) stiripentol does not act as a GABA receptor agonist but instead it inhibits the synaptosomal uptake of radiolabelled GABA; b) the effect of stiripentol is based on an inhibition of cytochrome P-450 isoenzymes involved in the hepatic catabolism of other antiepileptic drugs (inhibition by stiripentol of several isoenzymes, in particular 3A4, 1A2 and 2C19).

The development programme/Compliance with CHMP Guidance /Scientific Advice

The product was first identified by BIOCODEX, in 1978 with early clinical development starting in 1980s. Stiripentol has been used in clinical trials in other forms of epilepsy such as Lennox-Gastaut syndrome, and in preliminary studies that included all forms of epileptic syndromes. Amongst all these types of epilepsy, subjects with SMEI appeared to have the best response in open studies. Preliminary (uncontrolled) studies showed the potential utility of this agent in combination with other anti-epileptic agents (AEDs). Subsequent studies demonstrated efficacy in those less than 20 years of age and specifically in Lennox-Gastaut syndrome in combination with carbamazepine. The supportive data also includes a compassionate use study and post marketing data resulting from temporary authorisation in France for continued compassionate use in approximately 200 patients.
According to the applicant ethical guidelines prevalent at the time of conduct of studies have been followed as ICH/GCP guidance came into force after initial development of the product had taken place.
No formal CHMP scientific advice was sought or provided during the development of this product.

1.2 Quality aspects

Introduction

Stiripentol is one of a family of novel α-ethylene alcohols with activity in the central nervous system and was granted orphan drug status in 2001 for the treatment of severe myoclonic epilepsy in infancy. It is chemically unrelated to all currently marketed antiepileptic drugs. Diacomit is presented in two pharmaceutical dosage forms - capsules and powder for oral suspension (in unit dose sachets) each containing 250mg and 500mg of stiripentol as active ingredient.
The capsules are gelatine-based and are packaged in polypropylene bottles with polyethylene closures with a tamper-proof tear band. The powder is packaged in paper/Al/PE sachets.
The excipients for each presentation and the pack sizes are as defined in the SPC.

Drug Substance (to be changed in the EPAR to "Active Substance")

Stiripentol INN has an asymmetric carbon atom at the 3 position and hence has 2 enantiomers, but is produced as the racemate. It is a white to pale yellow crystalline powder, practically insoluble in water at 25°C. The log octanol/water partition-coefficient is 2.94. Stiripentol has not been observed to exhibit polymorphism.

- **Manufacture**

Stiripentol is synthesised in a simple two-stage process beginning with an aldol condensation and subsequent reduction of the resulting ketone. This condensation step is reported in literature to largely yield the trans isomer, whilst the reduction of the ketone to stiripentol is non-selective and yields the racemate. Confirmation that the trans isomer is initially formed has been provided by spectroscopic evidence. Very low levels of the cis-isomer have been detected in 10 batches. Satisfactory spectroscopic evidence has been provided to confirm the structure of the active substance which is routinely produced according to the defined synthetic process. The absence of polymorphism has been satisfactorily addressed by means of X-ray powder diffraction and DTA studies.

No formal process validation data are provided. It is argued that this is a two-step process manufactured with commercially available raw materials and batch data from 10 commercial batches demonstrate consistency of the process to produce product with desired specifications. This is accepted.
The organic impurities arising from synthesis and degradation and residual solvents have been investigated, and in general, impurities have been qualified with reference to relevant toxicological studies.

- Specification

Satisfactory descriptions are provided for analytical methods. Most are standard pharmacopoeia procedures. In-house methods are described for identification (HPLC, IR and colour reaction), related substances (HPLC), residual solvents (GC) and assay (HPLC).

Because of the low aqueous solubility, particle size is controlled by a laser light scattering method.

Batch analytical results for 10 pilot/production scale batches manufactured at the proposed manufacturing site have been provided. These data confirm compliance with the proposed specification. None of the named impurities in the impurity studies have been detected in batches so far.

- Stability of the Active Substance

Forced degradation studies show that stiripentol in the solid state is stable to high temperatures and light, and in solution it is stable to basic and oxidising conditions. Stiripentol as an aqueous suspension at pH 5 is stable at high temperatures. Stiripentol is also stable in a range of solvents (cyclohexane, toluene, methanol and ethanol) heated to reflux. However under acidic conditions, instability is noted.

Formal stability studies have been performed on production scale batches. Results for up to 24 months under long-term conditions (25°C/60% RH) and 12 months at accelerated conditions (40°C/75% RH) have been provided. Parameters monitored are appearance, identification (HPLC, IR), appearance of solution, loss on drying, degradation impurities and assay. The analytical methods are stability-indicating and are the same as those used for routine quality control.

Medicinal Products

1. Capsules

- Pharmaceutical Development

Studies have naturally focussed on the solid-state properties of the active substance, e.g. particle size control and polymorphism. Solid state active-excipient compatibility studies with a range of excipients under elevated temperatures has been investigated and no evidence of incompatibility was noted with the excipients finally selected. Starch and PVP were tested as binders and PVP selected as it yielded granules with good flow with minimal variation in density. Sodium starch glycolate is added as disintegrant and is incorporated intra- (1%) and extra-granularly (0.5%). Magnesium stearate added extra-granularly is used as lubricant at a level of 0.5%. Excipients used are standard pharmacopoeial ingredients for solid-dose preparations.
Stiripentol is practically insoluble in water, but is well absorbed following oral administration. Thus since the particle size is controlled, the rate determining step is likely to be dissolution. Originally a hydroalcoholic dissolution medium was developed for routine quality control although this was replaced with an aqueous sodium laurilsulphate solution which gives better discriminatory power between batches with different active substance particle sizes.

- Adventitious Agents

Magnesium stearate used is of vegetable origin, and EDQM Certificates of Suitability have been supplied for the gelatine used in the capsule shells.

- Manufacture of the Product

The manufacturing process is relatively straightforward and involves standard pharmaceutical unit operations: mixing, granulation, tray oven drying, screening, extra-granular blending and encapsulation before final packaging. The process and equipment have been adequately described. In-process controls are satisfactory for the processes described.

- Product Specification

The specification is relevant for a product of this type and includes validated tests for identity of active substance, assay (HPLC), uniformity of mass (Ph. Eur.), disintegration time, dissolution in an aqueous medium containing sodium laurilsulphate, and impurities (HPLC). Batch analytical profiles of three batches of each strength are provided and show satisfactory uniformity and compliance with the agreed specification, indicating that the process is under control.

- Stability of the Product

Stability Data are presented for up to 36 months at 25°C and 12 months at 40°C. Apart from one batch of the 500mg capsules that showed a reduction in disintegration time, no trends or significant changes in appearance, disintegration time, mean dissolution at 60 minutes, assay, named and unknown impurities are noted with all other batches. In the absence of a formal photostability study to confirm photostability of the finished product, a requirement to store in the original package/outer container is considered appropriate.

In total, the stability results generated so far support the shelf life and storage conditions as defined in the SPC.

2. Powder for Oral Suspension

- Pharmaceutical Development

The same granule formulation (i.e. active, PVP and portion of sodium starch glycolate) used for the capsule formulation has been chosen for development with additional excipients added to the external phase to obtain the final powder blend. The functions of added excipients are well-known and standard. Carmellose sodium and hydroxyethylcellulose, as viscosity modifying agents to ensure dispersion in a glass of water, dehydrated glucose syrup as diluent and aspartame as sweetener. Flavours and colour have been added to improve taste, appearance and to improve compliance.
The powder for oral suspension has been developed as an alternative to the capsule formulation, and therefore attention has focussed on demonstration of bioequivalence and interchangeability.

- Manufacture of the Product

The manufacture of the powder for oral suspension also follows standard pharmaceutical processes involving mixing, granulation, tray oven drying, screening/milling, extra granular blending, sachet filling and sealing before final packaging. In-process controls for the granule manufacture are basically the same as those for the capsules and these are satisfactory for the processes described.

- Product Specification

The specification is relevant for a product of this type and includes validated tests for identity of active substance, assay (HPLC), uniformity of mass of sachet contents (Ph. Eur.), and impurities (HPLC). In addition a test for dissolution has been added, considering the low solubility of the active substance. Batch analytical profiles of three batches of each strength are provided and show satisfactory uniformity and compliance with the agreed specification, indicating that the process is under control.

- Stability of the Product

Stability studies have been performed on two batches of the 250mg sachet and two batches of the 500mg packaged in the intended commercial packaging. The studies were carried out under conditions in accordance with ICH recommendations; at 25°C/60%RH for long-term testing and 40°C/75%RH for accelerated testing. Samples were tested for appearance, assay, impurities, dissolution and microbiological quality.

Stability results up to 48 months at 25°C and 12 months at 40°C are submitted. No trends or significant changes in appearance, assay, or impurities are noted on storage under real time or accelerated storage conditions. Microbial content is low and no changes are noted on storage.
The results generated so far support the shelf life and storage conditions as defined in the SPC.

Discussion on chemical, pharmaceutical and biological aspects

The synthesis and control of the active substance has been described in a satisfactory manner and the stability has been demonstrated. Concerning the two pharmaceutical forms, these are standard and the development, manufacture, and control of the formulations has been carried out in a satisfactory way, bearing in mind the low aqueous solubility of the active substance. Stability results allow suitable storage conditions and a realistic shelf life.

In all, the batch results generated so far indicate that the products are under good control with low batch variability, and should perform in a consistent manner in the clinic.

1.3 Non-clinical aspects

Introduction

Most of the non-clinical studies were conducted according to GLP guidelines. Those conducted before the start of GLP requirements (mainly embryo-foetal studies) were considered of adequate standard.

Pharmacology

- Primary pharmacodynamics

In vitro studies
According to the *in vitro* receptor binding study by Poisson et al, 1984 (published study), stiripentol did not show any affinity for GABA A or B, glycinergic or benzodiazepine receptors up to the µmolar range. In the same study, stiripentol was found to reduce synaptosomal GABA uptake (IC50 5x10-5) and to slightly increase (+22%) brain concentrations of GABA after "in vivo" administration (300 mg/kg i.p.).
In this respect, the R(+) enantiomer is thought to be the more active by a factor of about 2.

A recent publication (Quilichini et al 2006) reports effects of stiripentol on isolated GABA transmission in postnatal rat hippocampal neurons. Experiments were performed on hippocampal slices taken from Wistar rats between postnatal days 7 and 8. In this model, stiripentol markedly enhances GABA release and prolongs GABAA receptor-mediated currents. Stiripentol increases the mean open duration of GABAA receptor-dependent chloride channels by a barbiturate-like mechanism.

In Vivo Studies
Since no specific experimental models of SMEI exist, the models of generalized seizures used can be considered acceptable.
In a wide variety of *in vivo* models using mice, rats and primates stiripentol itself consistently showed anticonvulsant activity but with variability between models.
From the few studies in which the anticonvulsant activity of oral stiripentol was examined (in most cases the drug was administered i.p.), an ED_{50} of 300-800 mg/kg in mice and around 400 mg/kg in rats can be extrapolated.

Although stiripentol has been shown to inhibit slightly the uptake of glycine and GABA by synaptosomes this is thought to be only a minor component - if a component at all - of its antiepileptic activity in clinical use. The mechanism of the anticonvulsant effects of stiripentol remains unclear.

Interactions with other anticonvulsant drugs
The main component of the activity of stiripentol is considered to be the inhibitory effect on CYP450 enzymes which in combination therapy leads to reduced metabolism of other anticonvulsant drugs.

The ability of stiripentol to potentiate the activity of other anticonvulsants was evaluated. In the model of PTZ-induced seizures in mice, stiripentol (100-200 mg/kg i.p.) increased the anticonvulsant effects of low to moderate doses of valproate, diazepam, valproate + diazepam, valproate + phenobarbital. Some potentiation of the effects of phenytoin was observed in the model of electroshock in rats. The effects of carbamazepine were very slightly increased. No statistical analysis was performed.
Therefore, the proof of efficacy (through pharmacodynamic interactions) of stiripentol in combination with anticonvulsant drugs should be derived from the clinical data.

- Secondary pharmacodynamics and Safety pharmacology

Nervous system. The effects of stiripentol on the CNS were mainly the occurrence of sedation and ataxia in the same doses range as for the anticonvulsant effects. Stiripentol also markedly enhanced the central depressant effect of chlorpromazine, possibly based on its own central depressant effect rather than metabolic interaction.

At doses of 200 mg/kg i.p. or 150-200 mg/kg p.o., stiripentol reduces reserpine-induced palpebral ptosis. Such an effect was evident up to 3 hours after i.p. injection of stiripentol. Stiripentol 200 mg/kg i.p. did not potentiate amphetamine-induced stereotypies.
The possible anxiolytic effects of stiripentol were evaluated in the four-plate test in rats. Although stiripentol increased animals' activity at the dose of 50 mg/kg i.p. (an index of anxiolytic activity), the drug reduced the effects of diazepam in the same model.
Ethanol-induced narcosis was potentiated by both single and repeated (over 5 days) administration of 200-400 mg/kg i.p. stiripentol. The compound was however less potent than diazepam in this test. Narcosis induced by benzodiazepines was potentiated as well by stiripentol 100 and 200 mg/kg i.p.
The analgesic properties of stiripentol have not been demonstrated given the lack of a control group in the hot plate test and the absence of statistical analysis in studies on the potentiation of the analgesic effects of codeine and glafenine.

At doses of 250-1000 mg/kg p.o. in mice, stiripentol reduced and increased basal motor activity if administered 30 min-2 hours and 3-4 hours before the test. A dose-dependent depression of locomotor activity was observed in the open field test at doses of 50-200 mg/kg i.p. At 200 mg/kg i.p., a reduction in aggressiveness in mice was found. Up to the dose of 200 mg/kg, the drug did not influence the acquisition of a conditioned reflex or muscle tone in mice. In the rota-rod test, stiripentol was dose-dependently active at doses of 200-600 mg/kg i.p.
Hypothermia was induced in mice at 200 mg/kg i.p.

Cardiovascular effects. In rats, stiripentol 100 mg/kg i.p. did not affect capillary permeability or resistance. Administered at doses of 2.5, 5 and 10 mg/kg i.v. in dogs, reduced blood pressure and heart rate and increased vertebral artery flow and brain oxygen consumption.
Furthermore, no treatment-related electrocardiographic changes were observed in the repeat-dose toxicity studies up to 6 month in monkeys.
At the request of the CHMP, the applicant explained further the potential for cardiovascular effects. In the literature (Danielsson and al, 1998, 2003,2005), cardiovascular effects (and birth defects) due to activity on hERG channels are described for cation-channel active AEDs such as lamotrigine, phenytoin, phenobarbital and carbamazepine, but not for AEDs non-active on cation-channels such as gabapentin or valproic acid. Therefore, due to its mechanism of action, stiripentol, would not be expected to have such effects on the cardiovascular system. This is supported by the absence of cardio-vascular findings in the clinical trials.

Blood. Administered at doses of 100 mg/kg i.p., stiripentol did not affect clotting time in rabbits or bleeding time in guinea pigs.
Gastrointestinal tract. At doses of 200 mg/kg i.p. or 400 mg/kg p.o., stiripentol did not influence intestinal transit or faeces production in mice. No ulcerogenic effect was seen in rats given 2000 mg/kg p.o...

Urine and bile output. Stiripentol did not influence urine output up to 200 mg/kg i.p. or 750 mg/kg p.o. in rats. In the same species, bile output was not influenced by the intraduodenal administration of 100 mg/kg.
Endocrine system: no effects were observed at 200 mg/kg p.o.

Overall, there were no findings of potential clinical concern in the secondary pharmacodynamics and safety pharmacology programmes.

- Pharmacodynamic drug interactions

The influence of stiripentol on the blood levels and/or on the effects of several other drugs was studied.
Lidocaine, but not lithium levels were increased. Stiripentol did not influence the latency or the magnitude of the hypnogenic effects of halotane, nor the toxicity induced by digitoxin or imipramine.
Stiripentol did not potentiate dihydroergatamine-induced acute toxicity, on the contrary, it reduced its toxicity.

The effects of acenocoumarol and phenindione on prothrombin levels were potentiated by stiripentol. The hypoglycemic effects of glibenclamide were also potentiated. Salbutamol-induced tachycardia was slightly potentiated. Stiripentol enhanced the myorelaxation induced by diazepam.
Conversely there was no interaction with ethynyloestradiol, atenolol or labetalol.

Pharmacokinetics

The pharmacokinetics of stiripentol were determined by analysis of radioactivity after administration of 14C and 3H radiolabelled stiripentol, and by HPLC when unlabelled stiripentol was administered. Proton magnetic resonance (1H-NMR) and HPLC were employed to identify and determine the enantiomers of stiripentol.

Absorption
Following oral administration of the racemate, absorption in the monkey (21%) was less than that in the rat (60-70%). In the latter species the mechanisms underlying absorption from the GI tract included stereoselective processes that lead to an enrichment of the less active S(-) enantiomer in plasma.

The relationship between plasma levels and anticonvulsant effects of stiripentol was studied in rats. Stiripentol was administered i.v. (20 mg/kg) or p.o. (300, 600, 800 mg/kg), and then the animals were treated with PTZ (70 mg/kg s.c.) at different time points (up to 2 or 24 hours after i.v. and p.o. stiripentol, respectively). No formal calculation of the main pharmacokinetic parameters (Cmax, Tmax, AUC and t1/2) was done. After i.v. injection, the highest levels of stiripentol (25 µg/ml) were reached after 10 min, followed by a progressive decrease and then by a plateau at 4-6 hours. After oral administration, maximum plasma levels were not clearly dose-dependent: 34, 81 and 71 µg/ml following doses of 300, 600 and 800 mg/kg, respectively. At all doses, the maximal levels were reached around 8 hours after the administration. Measurable levels (around 25 µg/ml) were still present 24 hours after the administration of 600 and 800, but not 300 mg/kg.

Distribution
Distribution studies are limited to the rat, both pregnant and non-pregnant. Following oral administration, the highest concentrations were found in liver, adrenal gland and mammary gland. The brain concentrations were lower than those in blood in both animal groups. Measurable concentrations were found in fetuses, although they were approximately 2.7 folds lower than those in blood.

Metabolism
Zhang et al, 1990 showed that stiripentol (200 mg/kg p.o.) undergoes extensive metabolism to a series of products which are excreted by urine. In this regard, the metabolic fate of stiripentol in rats is similar to that in humans, since all the 13 urinary metabolites found in humans in previous studies are also present in rat urine.

The metabolism of stiripentol involves five metabolic pathways, (1) conjugation with glucuronic acid, (2) oxidative cleavage of the methylenedioxy ring, (3) O-methylation of catechol metabolites, (4) hydroxylation of the t-butyl group, and (5) conversion of the allylic alcohol side-chain to the isomeric 3-pentanone structure. Oxidative cleavage of the methylenedioxy ring generating catechol derivatives represented the major quantitative route of biotransformation in rats and humans. In contrast to humans, however, rats excreted a very little amount of the glucuronide conjugate, while displaying a greater ability to metabolise stiripentol by oxidative routes.

Studies on the P450-inhibitory profile of stiripentol.
The effects of stiripentol on different enzymatic activities have been studied in hepatic microsomes obtained from animals (rats and mice of both sexes) treated in vivo *with three different doses of the drug. Many enzymatic activities were induced by stiripentol at the highest dose (800 and 600 mg/kg in rats and mice, respectively), and, to a lesser extent, by the intermediate doses (220 and 200 mg/kg). The main effect concerned EROD activity, while phenacetin de-ethylase (another activity characterizing the CYP 1A2) was induced to a lesser extent. Some enzymes (such as those relevant to CYP 2C and CYP 2D6) were significantly inhibited. Important sex and species differences were observed. In general, the induction of enzymatic activities was weaker with respect to that of the reference compounds (phenobarbital and α-naphtoflavone).*
Mesnil et al, 1988 determined the effects of stiripentol on rat brain cytochrome P-450-mediated naphthalene hydroxylation *in vitro*. A concentration-dependent inhibition of the hydroxylation reaction was observed (IC_{50}: 1.21 μM). In an *ex-vivo* study, rats were administered with stiripentol 100 mg/kg i.p. and then sacrificed at different time points to measure the inhibition of naphthalene hydroxylation in microsomal fractions of brain homogenates. The highest inhibition (71%) was observed 2 hours after dosing.

Stiripentol both inhibits (rat brain cytochrome P450-mediated naphthalene hydroxylase inhibition) and induces (CYP1A2, 3A, 2C) enzyme activity.

Excretion
Tang et al, 1994 examined the faecal excretion of the stiripentol enantiomers after oral administration to rats. When the racemate was administered, approximately one third of the administered dose was found in faeces suggesting incomplete absorption. Faecal excretion was 14% and 4% of the total dose after administration of the S (-) and R(+) enantiomers, respectively. Irrespective of the enantiomer administered, faecal stiripentol consisted almost exclusively of the R(+) enantiomer. Stereoselective absorption and/or conversion within the gastrointestinal tract were proposed as possible mechanisms for this phenomenon.

Lin and Levy, 1983, showed that, in monkeys treated with different i.v. (40-120 mg/kg), oral (80 mg/kg) or i.p. (80 and 120 mg/kg) doses of stiripentol, the percentage of the dose excreted as glucuronide in urine was between 32.2 and 40.5 irrespective of the route of administration. More than 70% of the total dose was excreted in urine within 2 hours, 80% within 4 hours and 93% within 8 hours. The fraction of dose excreted unchanged in urine ranged between 0 and 3%.

The ability of stiripentol to pass into the milk was examined in lactating goats. Following a single or repeated doses (200 mg/kg p.o. over 7 days), stiripentol passed rapidly into the milk. A steady state was reached after the second administration and the milk/plasma ratio was around 1.

Toxicology

- Single dose toxicity

Stiripentol exhibited low acute toxicity as indicated in the table below:

Species	**Route of administration**	**LD50 (mg/kg)**
mouse	oral	3000-5000
mouse	iv	72-78
mouse	ip	ca.1500

rat	oral	>3000
rat	ip	1000-1500

The main findings were clinical signs consistent with effects on the CNS (agitation/sedation, hypothermia, convulsions, respiratory depression).

- Repeat-dose toxicity (with toxicokinetics)

Repeated-dose oral toxicity studies were conducted in mouse, rat and Cynomolgus monkey.
Mice were dosed for 13 weeks with 0, 60 and 800 mg/kg/day.
In the rat, two 6 month oral studies were conducted: the first, non-GLP, in the Wistar rat at doses of 0, 30, 60, 300 mg/kg/day and the second in the SD rat at doses of 0, 80, 220 and 800 mg/kg/day.
In monkeys, a 4-week study (from 100 to 900 mg/kg/day) and a 26-week study (100 to 600 mg/kg/day) were conducted.

The liver was a target organ in all three species. Increased liver weight with hepatocellular hypertrophy was a common finding at mid and high doses with reduced ALP and ALAT activities. These findings were interpreted as the consequence of an adaptive response to an increased "metabolic load" as evidenced by significant liver microsomal enzyme induction at doses > 200 mg/kg/day in rodents (Guyomard and Chesné, 1994).
The kidney was also a target organ in rats and monkeys with signs of tubular nephrosis at high doses. However, the clinical relevance of these findings is considered to be unlikely.
The NOEL (no observed effect level) was defined as 80mg/kg in rats and 100 mg/kg in monkeys corresponding to a Cmax of 5-10 µg/ml in plasma.

- Genotoxicity

A complete programme comprising bacterial and mammalian cell mutation tests (Ames, V79) clastogenicity tests (chromosome aberration in CHO cells and human lymphocytes), *in vitro* UDS assay in rodents hepatocytes and an *in vivo* mouse micronucleus test an were conducted.
Stiripentol was clastogenic at cytotoxic concentrations only (CHO cells). All the other tests were negative although the Ames test was limited in terms of strains and species used (E. coli and Salmonella TA 102 not included). Overall, there was no evidence of genotoxicity.

- Carcinogenicity

Carcinogenicity studies were conducted in mice (78 weeks) and rats (102 weeks).

In the **mouse** study there was an increase in the incidence of hepatocellular adenomas and carcinomas secondary to hepatocellular hypertrophy in the mid- and high-dose groups as shown below:

Parameter	Control 1*		60 mg/kg/day		200 mg/kg/day		600 mg/kg/day	
	Male	Female	Male	Female	Male	Female	Male	Female
Animals/group	50	50	50	50	50	50	50	50
Body weight gain W –2 to W 78 (%, relative to control)	-	-	-5.3	0	0	-11.6	-15.8	-17.5
Survival at 78 w (%)	88	72	70	86	84	76	68	76
Animals with neoplastic liver lesions (no/group)	2	1	6	1	6	5	11	18
[1]Hepatocellular adenoma (no/group)	4/50	0	4/49	0	11/50	3/50	9/50	6/50
[2]Hepatocellular carcinoma (no/group)	1/50	1/49	1/49	0	6/50	0	6/50	15/50
C_{max} at 78 w (µg/mL)	ND	ND	19.6	14.3	31.9	40.4	41.1	59.6
[3]Exposure margin	-	-	1.2	0.9	1.9	2.4	2.5	3.6

[1]and [2]: P<0.005 vs. control (Peto trend test, one-tailed). [3]: Exposure margin was calculated with respect to an estimated adult human exposure of 16.5 µg/ml after 50 mg/kg.

The applicant pointed out that the liver tumours arise as the result of an epigenetic mechanism and such findings have been well-documented following the administration of enzyme inducers such as stiripentol to mice.

Further rationalisation is supported by published data suggesting that hepatocellular neoplasia in mice associated with metabolic activation/phenobarbitol-like promotion is of limited significance with regard to human safety. Among them, a statement in the ICH Guideline S1B: '*the high susceptibility of mouse liver to nongenotoxic chemicals has been the subject of many symposia and workshops. These have concluded that these tumours may not always have relevance to carcinogenic risk to humans and can potentially be misleading*'

In the rat carcinogenicity, there were also adverse hepatic effects (centrilobular hepatocellular hypertrophy) but no evidence of increased frequency of tumour formation in the liver or in any other organ.

Notwithstanding the above explanations of the adverse findings in the mouse study, the lack of any exposure margin at the NOEL remained a concern for CHMP since extrapolation to man on this basis would lead to an ineffective clinical dose. At the request of the CHMP, the applicant further addressed in more details the mouse oncogenicity results. The CHMP concluded that, considering the relatively weak oncogenic potential and the known mechanism, together with the proposed indication, the risk:benefit would be acceptable with a suitable statement in the SPC.

- Reproduction Toxicity

Reproductive and developmental toxicity has been assessed in rats, mice and rabbits.

In the fertility and early embryonic development, apart from non-specific signs such as delayed ossification and increased pup mortality at the high dose (800mg/kg), there was no indication of adverse effects on fertility, embryo-fetal development and post-natal development.

In embryo-fetal development studies in mice, the increased incidence of cleft palate observed in one study at 200 and 300 mg/kg/day, was not confirmed in further studies in mice (5 in total). There was also no evidence of teratogenicity in a rabbit study.
Although the initial positive study is probably of no relevance to the infant patient population, this finding is mentioned in the SPC.

Finally, in the second of two pre- and post-natal studies in the rat, there was increased mortality in both dams and pups. There was no evidence of teratogenicity in the first study in which dams were dosed during the entire period of organogenesis.
This effect on pup viability is not an unusual finding with CNS-active agents. This is not considered to be relevant to the infant patient population.

- Toxicokinetic data

Limited toxicokinetic data are available from a few studies consisting in values for Cmax only (usually 1-2 hours post-dosing) and not AUC. In general, safety margins compared to humans (concentration of 16.5 µg/ml after 50 mg/kg) are low or non-existent (1 to 3.5x).

- Other toxicity studies

There was no evidence of immunotoxicity in the repeated-dose toxicity studies.
A single dose study with impurities did not induce any signs of toxicity or mortality.

Ecotoxicity/environmental risk assessment

The calculation of the $PEC_{surface\ water}$ resulted in a value below the trigger value of 0.01 µg/l defined in the CHMP guideline (CHMP/SWP/4447/00). The calculation was based on a refined Fpen of

0.00034% considering the market penetration as a third of the incidence of the disease (0.1/10000). The daily dose of 2g/patient was assumed.
The product having a low Log Kow, does not have potential bioaccumulative properties. Furthermore the extensive metabolism into readily biodegradable catechol or similar metabolites further reduces the potential risks to the environment.
It was therefore concluded that the use of stiripentol in SMEI patients does not represent a risk for the environment and do not require specific labelling for the environment.

Discussion on the non-clinical aspects

Pharmacology
In vitro and in vivo experiments demonstrated that stiripentol itself has pharmacodynamic activity consistent with a potential therapeutic effect in the proposed application. This consisted of inhibition of glycine and GABA uptake by synaptosomes. The R(+) enantiomer was more potent by a factor of two.

This however, is probably a minor component of the anticonvulsant activity of stiripentol, which is considered to result mainly from the inhibition of enzymes responsible for the metabolism of existing anti-epileptic medications.
There were no findings of clinical concern in a full programme of safety pharmacology studies.

Pharmacokinetics
The ADME profile of stiripentol has been adequately characterised.
Stereoselective processes in the GI tract lead to an enrichment of the S(-) enantiomer in plasma. Stiripentol both inhibits (rat brain cytochrome P450-mediated naphthalene hydroxylase inhibition) and induces (CYP1A2, 3A, 2C) enzyme activity.

The few toxicokinetic data indicate that safety margins with respect to adverse effects observed in toxicity studies are low or non-existent. Unfortunately, these are based on Cmax, there being no measurement of AUC. Nevertheless, there are no issues of potential clinical concern.

Toxicology
A full programme of toxicity studies has been submitted. The only finding of potential clinical concern was the formation of hepatocellular adenomas and carcinomas in the mouse carcinogenicity study. In spite of the lack of any exposure margin at the NOEL, the CHMP concluded that, considering the relatively weak oncogenic potential and the known mechanism, together with the proposed indication, the risk:benefit was acceptable with a suitable statement in the SPC.

1.4 Clinical aspects

Introduction

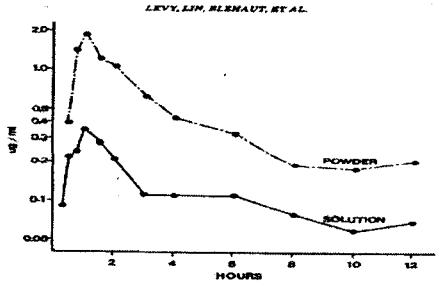
The development programme has lasted ~25 years and a number of clinical studies were initiated before the current ICH/GCP guidelines came into force. However, according to the applicant, the studies followed all ethical guidelines in practice at the time of conduct of the studies.

Pharmacokinetics

The majority of the kinetic studies with Stiripentol were conducted in the 1980s and these have been appended as publications (papers) or summary study reports. Detailed study analyses are not available for a large majority.
There are four main pharmacokinetic studies in healthy volunteers (Levy 1893 and 1984b, STI UNI study) conducted in the 1980s to determine the bioavailability, non-linearity and enantiomer metabolism (BC 287) included in the dossier. Other studies are either in epileptics or healthy volunteer (HV) for interaction, efficacy or safety where-in kinetics have been assessed (Levy 1984a, Levy 1985, Kerr 1991, etc).

Stiripentol has not been studied in monotherapy.

- Absorption



The bioavailability of stiripentol is variable and is apparently dependent on the formulation. The powder or granules for suspension have lower bioavailability (21±9%) relative to the capsule. It should be noted that the active agent has a significant degree of degradation in acid environment
(pH 1.0). Due to the lack of bioavailability of stiripentol between formulations (powder/granules for suspension and capsules), the applicant committed to conduct a bioequivalence study between the capsule and the powder /sachet formulations as part of post-authorisation a specific obligation.

- Distribution and elimination

Stiripentol has a large volume of distribution and a dose disproportional clearance. A dose increase from 600 to 1200mg/d resulted in 235% rise in Css while a dose increase from 1200 to 2400mg/d was associated 397% rise. The oral clearance was 41.5±23.4 l/d/kg (600mg), 20.3±8.8l/d/kg (1200mg) and 8.5±3.8l/kg/d (2400mg) respectively. A significantly large intersubject variability was noted (300-400%). There are limited data in children regarding distribution and elimination and it is unknown whether anticonvulsants with high protein binding affinity will displace stiripentol (99% bound) from plasma proteins influencing safety and efficacy.

- Metabolism

Stiripentol is a racemate (administered in the clinical studies) with enantiomers that are interconvertible, albeit at different rates. The proportion of interconversion varies depending on the enantiomer administered. These may have a bearing on the administration of racemate as R enantiomer is more potent. However in the clinical programme, there is neither evidence that interconversion is affected by non-linearity of metabolism nor a particular enantiomer is related to adverse events or safety.

In man, stiripentol is metabolised extensively, with 13 urinary metabolites accounting for ~70% of the oral dose. There are no active metabolites identified in man.

- Dose proportionality and time dependencies

The kinetics and linearity of Stiripentol were studied in two main studies; Levy et al 1983 and STIUNI study.
Both compartmental and non-compartmental approaches were used for calculation of kinetic parameters (Cmax, AUC, AUCinf, T1/2, Tmax, and MRT). With the compartmental model, the t1/2 could not be determined after 500 mg dose, but with the half-life independent model, this was possible.

Table-4a: *Kinetics in a two-compartment model. model*

	500mg (n=6)	1000mg (n=12)	2000mg (n=12)
C_{max}STP	3.1 ± 0.9	7.1 ±1.9	13.2 ±3.6
T_{max}	2.7 ±1.0	2.7 ±1.3	3.3±1.0
$T_{1/2}$ β	4.4 ±2.1	10.1 ± 3.3	13.7 ± 6.2
$AUC_{0\text{-}inf}$	9.9± 3.4	31.1 ±10.9	87.7 ±27.7
Lag time	0.87 ±0.55	0.51 ±0.46	0.48±0.33

Table -4b: *Kinetics in a non-compartmental model;*

	500mg	1000mg	2000mg
C_{max}STP	2.63 ±1.18	6.63 ±1.83	13.8 ±4.83
T_{max}	2.342 ±0.76	2.42 ±1.00	2.96 ±1.01
$T_{1/2}$ β		7.82 ±1.86	11.0 ±4.18
$AUC_{0\text{-}30H}$	8.85 ±3.77	32.1 ±10.7	79.0±24.2
MRT		7.67 ±1.79	11.1 ±2.94

It is believed that the non-linearity is predominantly due to 'Zero-order' absorption process with extensive hepatic metabolism. The exact reason for 'zero-order' absorption such as a transporter or active mechanism has not been identified. The zero-order absorption does not fully account for the dose-disproportional clearance and variability. Cmax is linear with dose; AUC may be higher at highest dose due to slight saturation of metabolic clearance.

- Special populations

Special population such as elderly, pregnant women, those with renal or hepatic impairment have not been specifically studied.

The absence of data in the elderly might be acceptable as the indication sought is primarily in children and use in the elderly is not anticipated. Similarly, renal dysfunction may be unlikely to significantly affect kinetics of stiripentol which is extensively metabolised in the liver.
However 70% of the administered dose is excreted as metabolites in the urine and renal impairment may affect this.

The absence of any data on the kinetics of stiripentol in those with impaired hepatic function and secondly, absence of information on hepatic function assessment in those receiving stiripentol are a concern as a significant effect and interaction with other AEDs metabolised by the liver (CYP450 enzymes) could be expected in such patients.
Due to the lack of data on the kinetics in patients with impaired hepatic and or renal function, the use of stiripentol in these patients is not recommended and this is reflected in the SPC.

Children
There is limited information/data regarding the distribution kinetics of stiripentol specifically in children. Considering that the predominant use of stiripentol is expected to be in children, the applicant, upon request of the CHMP, committed to perform a specific population pharmacokinetic study in children in order to establish this as a post-authorisation follow-up measure.

- Pharmacokinetic interaction studies

Stiripentol inhibits several CYP450 isoforms (3A4, 2C19, 2C9, 2D6 and 1A2). These have been assessed in one *in vitro* study and one *in vivo* study.
Interactions with AEDs such as carbamazepine, phenytoin, phenobarbital, clobazam and valproate have been assessed as combinations with an arbitrary dose reduction of 50% (25-75% range) recommended for all agents when combined with stiripentol. As several of these agents were administered simultaneously in a number of studies, it is not possible to differentiate individual interactions.
Studies are lacking regarding consequences of genetic polymorphism of these CYP isoforms on the interactions. No data on consequences of polymorphism of enzyme-pathways involved in stiripentol metabolism are provided either. Interactions with other drugs (non-anticonvulsant products) have not been studied in details although were partially explored in the initial *in vitro* study.
Major interactions are with other anti-epileptic drugs (anticonvulsants). Overall, stiripentol appears to potentiate other AEDs in controlling seizure activity. In the Kerr et al study, stiripentol reduced carbamazepine (CBZ) dose requirements by inhibiting clearance by 50±16%.
Tran et al in 1996 confirmed these and provided a regression equation describing the effect of stiripentol on CBZ-epoxide.
Farwell et al (1993), studied effects of addition of stiripentol to a combination of AEDs over a 24-week period and reported a decrease of seizure activity on average by 70% (5-95%).
Levy et al (1984) and subsequent studies using a combination of Stiripentol with phenytoin (steady dose) suggested a wide range of dose reductions (25-66%).
Other studies or trials show that addition of stiripentol to constant dose of PB (Phenobarbital) raised steady state concentration variably between 8-80% (mean 44%). Individual interactions may have provided a better understanding of pharmacology but poses a clinical problem in SMEI wherein the seizure activity is resistant to multiple drugs.

Pharmacodynamics

- Mechanism of action

The anticonvulsant activity of stiripentol was primarily investigated in animal models and subsequently studied in man in short term and long term clinical studies.

Putative antiepileptic mechanisms have been attributed to the following factors;

- to enhancing the central synaptic availability of GABA by inhibition of GABA metabolism and possibly, inhibition of synaptic GABA reuptake.

- stiripentol's inhibitory effect on CYP450 isozymes (1A2, 2C19, 2C9, 2D6 and 3A4) contributes to its antiepileptic action by enhancement of plasma levels of co-administered AEDs.

- Primary and Secondary pharmacology

A true relationship between plasma concentration and effect has not been studied as there are no monotherapy studies and intravenous preparations of stiripentol are not available for human use. Three studies in adult epileptics (Levy 1984, n=6; Kerr 1991, n=7 and Tran 1996, n=16), provide some information about different doses of stiripentol used. However, the clinical effect of these doses in terms of reduction in seizure activity has not been systematically examined (or published). Whilst plasma concentrations were determined in all studies, the concentration with most antiepileptic activity remains unclear. Tran et al in 1996 found that 7 mg/L of stiripentol (plasma level) was required for effects on carbamazepine metabolism. Whether other AEDs are affected by such a level or similar level is unclear.

Secondary pharmacology: The effect of stiripentol on the cardiovascular and digestive systems is difficult to assess in the absence of placebo-controlled studies. In the pivotal and long-term open trials, there were no major cardiovascular adverse events. However, effects on the digestive system were diverse and significant, although not systematically documented. For example, vomiting, weight-loss, and anorexia are consistently seen and in the clinical trials, significant weight loss was noted by many individuals. The mechanism of these effects remains unknown.

CYP450 enzyme induction or inhibition: Stiripentol is a potent inhibitor of CYP 450 isozymes in the liver and brain. It is likely that stiripentol inhibits CYP isoforms in all tissues. There is thus potentiation of all other commonly used anticonvulsants. Data on interactions with other agents are limited. Three studies in adult epileptics (Levy 1984, Kerr 1991 and Tran 1996) provide some information about different doses of stiripentol used. But these data are limited. The applicant committed to perform an *in vitro* study as a post-authorisation follow-up measure, to explore the enzymes involved.

Clinical efficacy

The development program included two pivotal efficacy trials conducted in the target population, SMEI. Preliminary data for efficacy of stiripentol in SMEI comes from one single study, the STEV where 25 of the 233 patients were diagnosed with this condition.
Overall there are two pivotal trials (65 patients), 4 supporting studies and 3 other open studies that assessed efficacy of stiripentol in all forms of epilepsy. Of these, data regarding the target population is available only from 2 pivotal, 1 supportive (STEV) and one open study (STILON). These studies are summarised in the table below:

Summary table of Efficacy studies.

Study ID	Design	Study	Other	Subjs by	Duration	Age	Diagnosis	Primary

		Posology	meds	arm entred/ compl.		(years)	Incl. criteria	Endpoint
Pivotal studies.								
STICLO-FR BC-299	DB, Rand, Placebo, multicentre studies	STP 50/mg/kg	VPA and Clobazam	N=41;	=3 months; 1 mth baseline	3-18 years	SMEI-specific (Dravet 1982), at least 4 fits /month	50% Seizure reduction
STICLO-IT BC-385		STP 50/mg/kg	VPA and Clobazam	N=24 23 compl	2 month DB			
Supportive studies								
Martinez-Lage 1986; BC-244 Phase-II	Open, phased study	2700-3000mg/day	CBZ (n=17), PHT (=2) and PB (=7)	N=29; n=12 on STP only; N=27 for STP+AED	8 wk baseline, STP replacement - over 8 weeks	16-57 years (30.5±9.7); 9 women	Mono or Bi therapy-complex partial seizures.	STP failed in AED resistant epilepsy
STEV study BC-288	Phase-II, Prospective, SB,	60mg and (d0-28) 90mg/kg/day then on		233 Pts	4 wks Ph-1; 12wk-STP phase	2-15 years (120males)	Children with refractory epilepsy	Change in Seizure freq
L& G study BC-274	SB; triphasic	65-83mg/kg/d	CBZ	N=24; 10 Females	60 days	1-22 yrs	Lennox-Gastaut Synd	⇓ fits in 72%;
STICAR-BC246	DB, Multi-centre, Phase III	2000mg/day	CBZ	~130		<20 years	>30kg,	
Open Studies								
STISEVR	Phased, DB	variable	CBZ	N=67			Partial seizures	32 responders, 17 to STP
Rascol, 1989	Open, Pilot,	1500mg/day	CBZ 700±49mg, PB=5, VPA=3,	N=7, (5 men)	4 months FU	Adults; 21-57 yrs	Complex partial seizures	⇓ fits 12.9±4.2 to 2.7±1 at 4mth
STILON BC-387	Open label, study in France; Observational,	4000mg/day	As required	N=155	3-5 years	Compassionate use, all responders from STICAR, WOW, Lennox, STEV, STIVER and STICLO		

- Dose response studies

Controlled monotherapy dose response studies with Stiripentol are not available. Very few studies indeed examined this aspect although different doses were used in different studies. Dose response studies in the target population (SMEI) have not been conducted. Many of the earlier reports and studies used fixed doses of stiripentol. The 1990 STICAR study, (adults or adolescents of at least 30Kg) used a fixed dose of 2000mg/day. In the WOW study, (patients aged 15-65 years) a fixed dose of 3000mg/day was used. The first attempt at using incremental doses came from Lennox-Gastaut study (1993, BC-274, patients aged 1-22 years) where doses higher than 50mg/kg with the highest ranging from 75-83mg/kg were used.

Studies where some dose response data may be extracted;

Study	Comedication	STP (stiripentol) dose (/day)	Comment.
Levy et al, 1985;	CBZ	2400-3000mg- 10 wks	Mean CBZ Cl fell fr 6.1±1.1 to 2.0 ±0.7L/h
Levy et al 1987	Carbamazepine	1000-3000mg- 2 wks	Fall in Cl; CBZE/CBZ ratio ⇓
Levy 1984,	5 PHT, 3 PB, 2 CBZ, and 1 Clobazam	600, 1200 & 2400mg	Mean phenytoin Cl reduced from 29.5±13.4 to 6.48±2.6
Kerr 1991	Carbamazepine	1500-3000mg	CBZ Cl reduced; Adults 1.25±0.25 to 0.61 ±0.14
Tran 1996	CBZ ± other AED	60mg/kg- 4weeks 90mg/kg- 8 weeks	CBZ metab maximally affected at STP 7mg/L plasma level (CBZE/CBZ ratio)

STEV study	Other AEDS	60mg/kg and 90mg/kg/day	Seizure reduction-modest.

The above studies however provide insufficient information regarding variations in dosage and the hence definite conclusions may not be drawn based on these. As there are no intravenous studies, and oral administration is believed to exhibit 'Zero order absorption' at high doses, true dose response is difficult to assess and remains unknown. In the STICLO studies (Pivotal), a fixed dose of 50 mg/kg/day was adopted. The basis for selection of this dose is unclear from the above studies. It is only in the STICLO studies (as discussed below) that the best evidence of efficacy of any dose is seen. Consequently, 50 mg/kg/day is the only recommended dose proposed in the SPC.

- Main studies

There are two main pivotal studies (STICLO-France and STICLO-Italy) that included the target population of SMEI and had identical protocol designs enabling some comparison and pooling of data. Due to the rarity of the target condition, the numbers included in each study were small (42 and 24 respectively). In the first STICLO-France study, these were only the preliminary or pilot numbers and in view of the significant difference between treatments, the data monitoring board decided to terminate the study without additional recruitment.

METHODS

Both studies, STICLO-France and STICLO-Italy, were double blind, multicentre, placebo-controlled and randomised, lasting about 3 months.

Study participants: The participants who were 3-18 years old, with diagnosed SMEI, and at least 4 tonic-clonic seizures per month were included. Additionally they had to be receiving clobazam (max 20mg/day) and valproic acid (≤30mg/kg/day), possibly receiving progabide or per-rectal diazepam. Doses other than these were altered to bring them in line with this protocol.

Design of the studies

The attached scheme displays the trial sequence used (Fig-4). The VPA (valproic acid) dose of 15 mg/kg/day during baseline was subsequently amended to ≤ 30mg/kg/day (by protocol amendment). This was done to counteract the poor control of seizures and therefore raises the possibility of baseline imbalance – influencing the results. In both trials, the comparison period was only two months.

The follow up period of open label stiripentol then continued on to the STILON study, assessing long term use of stiripentol in these children.

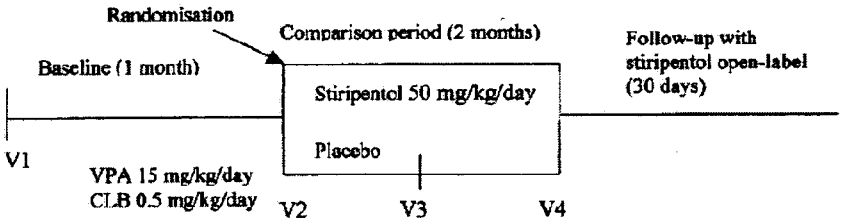


Fig-4; Study design of STICLO studies (Fr and IT)

The participant selection and design of the study (baseline and treatment period of 4 and 8 weeks) appear acceptable and in accordance with standard trials for anti-epileptic drugs. A longer period of treatment would have provided better clues towards the long-term efficacy of stiripentol. (at least 12 weeks as per CHMP guidelines).

Treatments: Stiripentol dose was fixed at 50 mg/kg/day. The rationale for this dose is however not specifically supported by the STEV study findings that used 60 and 90 mg/kg/day in children.

Moreover, the choice of the concomitant medications appears a little arbitrary as it is unclear from the STEV study results or protocol if there was a significantly better response in the valproate & clobazam groups. The choice of the anti-epileptics permitted is in line with the clinical management of this condition.

Objectives: