SCIENTIFIC DISCUSSION

1 Introduction

The Lennox-Gastaut syndrome (LGS) is a rare and one of the most severe forms of childhood epilepsy syndrome. The syndrome usually affects children between the ages of 1 and 8 years (typically between 3 and 5 years), but occasionally has its onset in children who are more than 8 years old. LGS begins in childhood but continues to manifest into adulthood in a large number of patients and has a significant morbidity and mortality. The hallmarks of the disease include the following triad:

- The presence of multiple seizure types: the most characteristic are tonic-atonic seizures and atypical absences, but tonic-clonic, myoclonic, and partial seizures are also frequently present. Tonic-atonic seizures often provoke sudden falls (commonly called drop attacks) and result in injuries.
- The presence of generalized discharges with slow spike-and-wave complexes in the electroencephalogram (EEG).
- The presence of mental retardation or a learning disability. In general, this is represented by a static encephalopathy, although the mental status may worsen in the course of the disease due to multiple causes, such as very frequent occurrence of seizures, sometimes subclinical, frequent head trauma from the falls associated with seizures (drop attacks), and undesirable cognitive effects of the high doses of antiepileptic drugs (AEDs) used to treat this very refractory type of epilepsy.

The aetiology of LGS remains unidentified in about half of the cases (cryptogenic LGS), whereas in others, the syndrome results from obvious brain injury (symptomatic LGS). The most common identifiable factor is a history of infantile spasms, occurring in up to one-third of the cases. Other causes include perinatal central nervous system trauma, meningitis and encephalitis, tumour, and severe head trauma. However, the electroclinical features are identical in cryptogenic and symptomatic LGS.
LGS accounts for approximately 1% of all new cases of epilepsy, although it may account for as many as 10% of the cases of severe epilepsy. Within European populations, the prevalence of LGS is 0.9 per 10,000 population across all age groups, and 0.7, 1 and 2 per 10,000 population in age groups between 0 and 19 years (EMEA/COMP Summary Report on an application for Orphan Medicinal Product Designation, COMP/390/03, 2004).

The long-term prognosis of LGS is frequently poor, with deteriorating mental function and persistently high rates of seizures. It is considered as an epileptic encephalopathy since epileptic phenomenon contributes to worsen the children condition. Seizure-free recovery is rare; fewer than 10% of patients became seizure-free in most reported series. Remission with preserved mentation occurs in very few patients; rather, IQ tends to deteriorate with age, and tonic seizures persist, but the slow spike-and-wave pattern does tend to resolve. Psychomotor delay and neuropsychiatric symptoms occur in 90% of LGS patients. Prolonged reaction time and information processing are the most impaired cognitive functions, which explains why these patients have a slow behaviour and are often rejected from school, even if intellectual capacity remains. Behavioural abnormalities occur in half the cases, including hyperactivity (most commonly), emotional instability, aggressiveness, destructive behaviour, autism, and antisocial personality. Such abnormalities and the arrest of educational progress are more prominent in older children and adolescents than in younger children. Chronic psychosis with episodes of acute exacerbation may also occur.
The mortality rate is difficult to assess: about 3% in the series of Gastaut, with a mean follow-up of 8 years and 7 months, and 7% in that of Loubier with a mean follow-up of 9 years and 9 months.

Current management of LGS is not satisfactory because the seizures associated with LGS are frequently unresponsive to standard anticonvulsants, in particular carbamazepine, phenytoin, and barbiturates. Valproate, often used as the drug of first choice, may help in controlling some seizure types, particularly atypical absence and myoclonic seizures. However, no controlled trials demonstrating its efficacy in LGS have been published: Benzodiazepines (clonazepam, clobazam, and

nitrazepam) are also frequently used as adjuvant intermittent treatment for LGS patients who have clusters of seizures. Nevertheless, no formal studies with these drugs have been reported. Newer AEDs, i.e., lamotrigine, felbamate and topiramate may be of benefit in patients with LGS. In one randomised clinical trial dedicated to LGS (adults and children mixed) for each of these compounds, the percentage change in tonic-atonic seizures or drop attacks with these AEDs has reportedly been about 25% greater than that seen with placebo A small number of patients became seizure free. In addition to their moderate efficacy, they are associated with potentially severe adverse reactions (aplasia and hepatitis with felbamate, skin rash with lamotrigine, cognitive disorders with topiramate).
Typically, either a benzodiazepine or one of the newer AEDs (lamotrigine, topiramate or felbamate) is used as add-on treatment, generally with valproate. Treatment with benzodiazepines, however, has sometimes been reported to worsen LGS and even to induce status epilepticus. The treatment of patients with LGS often involves polytherapy due to the lack of full response to any single AED. Even when a drug is initially effective, this may not persist long term. Nonetheless, patients usually benefit only minor improvements in seizure frequency and severity, as treatment success is uncommon in this condition.
Evaluation for a surgical approach (corpus callosotomy) is warranted in patients who are refractory to all these drug therapies. Surgery is purely palliative, however, and seldom produces total control of seizures.
Non-drug treatments for LGS, including ketogenic diet, vagal nerve stimulation for intractable drop attacks are used occasionally.

Rufinamide is a triazole derivative used as an antiepileptic drug (AED), structurally unrelated to currently marketed AEDs.

The primary *in vitro* pharmacodynamic data indicate that interacts with the inactivated state of the sodium channel and slows conversion to the active state thereby reducing the frequency of sodium-dependent action potentials in rat neurons, an effect that could contribute to blocking the spread of seizure activity from an epileptogenic focus.
Evidence from *in vivo* studies using animal seizure models showed that rufinamide is active in a broad range of animal models of epilepsy. No tolerance was observed in animals after repeated dosing.

Ciba-Geigy in Europe initiated the earliest clinical studies with rufinamide in 1987. Novartis, (merging of Ciba-Geigy and Sandoz), continued the development until 2001. Eisai Company, Limited. acquired the worldwide development rights to rufinamide from Novartis on 6 February 2004 for the submitted indication and further development work since this date has been carried out by Eisai.

Rufinamide was designated as Orphan in this indication by the Committee on Orphan Medicinal Products (COMP) (EMEA/OD/047/04) on 9th September 2004, adopted by the European Commission on 20th October 2004 (EU/3/04/240). It was designated as an Orphan on the basis that although satisfactory methods of treatment of LGS have been authorized in the Community, justifications have been provided to the COMP that rufinamide may be of significant benefit to those affected by the condition.
No protocol assistance in this indication was requested by the applicant.

2 Quality aspects

Introduction
Inovelon is presented as film-coated tablets containing 100, 200 or 400 mg rufinamide. The tablets are pink, 'ovaloid' in shape, slightly convex, scored on both sides. Each strength is identified by an embossed marking "€ 261", "€ 262" and "€ 263" only on one side of the tablet, for the 100, 200, and 400 mg strength, respectively.
Inovelon tablets are packaged in push-through PA/AL/PVC blister pack, also referred to as Alu/Alu blister pack in pack sizes of 10, 30, 50, 60 100 film-coated tablets for all strengths, whereas for the 400 mg strength only, an additional pack size of 200 tablets also exists.

Active Substance

Rufinamide, a triazole derivative, is the INN name of the active substance 1-(2, 6-difluoro-phenyl) methyl-1*H*-1, 2, 3-triazole-4-carboxamide. The molecular formula is $C_{10}H_8F_2N_4O$ and the Relative Molecular Mass 238.2. It appears as a fine, white, odourless and slightly bitter, not hygroscopic powder of needle shaped crystals with aggregates. It is practically insoluble in water, slightly soluble in methanol and very slightly soluble in ethanol. Due to the needle shaped crystals, the drug substance has a low bulk density, poor flow properties and strong tendency to agglomerate. The partition coefficient, log $P_{o/w}$ is 0.65, and the melting range is between 233°C and 238°C. There are four known polymorphic forms, A, A', B and C; A being the thermodynamically stable form.

- Manufacture

The synthesis of the rufinamide drug substance is a 7-stage process. For an individual step, several batches may be combined for workup. If a batch of an intermediate or the drug substance fails to meet specifications, the material may be reworked according to the procedure described in the synthetic description, starting at an appropriate stage. The product is isolated and purified by several steps of re-crystallisation initially with 2-propanol and later with methanol.
The manufacturing process of rufinamide is adequately controlled by the testing of the intermediates.
Rufinamide manufactured by the current process is exclusively modification A, which is the thermodynamically stable form. All batches of drug substance used for technical, toxicological and clinical investigations show modification A. There is no evidence of hydrate formation from differential scanning calorimetry (DSC) or from the stoichiometry of the molecule.
Batch analysis data from nine batches were presented and the consistency of the results confirm that the active substance can be manufactured reproducibly as all results are well within limits.

- Specification

The specification for control of the drug substance includes tests for appearance, bulk density (USP), crystal modification (X-ray diffraction), identification (IR, HPLC), clarity (Ph. Eur.) and absorbance (UV) of 0.1 % MeOH solution, heavy metals, sulphated ash (Ph. Eur.), assay (HPLC), related substances (HPLC) and residual solvents (GC). In addition to this control imposed by the manufacturer, Eisai performs a test for particle size distribution and bulk density, keeping in mind the very low solubility and poor flowability of rufinamide.
Additional tests for residual solvents (GC, CE) and loss on drying (Ph. Eur.) are included in the specification for rufinamide batches that are reworked.
Batches analysis provided, including batches used in pre-clinical and clinical studies, confirm the suitability of the specifications.

- Stability

Since the original development was dane by Ciba/Geigy/Novartis, stability studies have been performed by both Novartis and Eisai.
Novartis has performed long-term, accelerated and photo stability tests, stress testing and forced decomposition studies under different conditions. Results from these Novartis studies were presented for three batches as primary data. This data package contains results from long term testing, at 25°C/60 %RH and 30°C/70 %RH for up to 3 years. For accelerated testing, 40°C/75 %RH, results were presented for up to 6 months. The drug substance has additionally been subjected to photostability testing in accordance with ICH Q1B Guideline and has been found not to be photolabile. The drug substance was subjected to extremes of temperature (50°C to 100°C) and found to be stable.

Eisai has also performed stability studies on four commercial size batches of rufinamide. These batches have been put in on-going stability and results for up to 6 months were presented, demonstrating no significant change in any tested parameter after storage at 25°C/60 %RH, 30°C/70 %RH or 40°C/75 %RH.

In conclusion, the results from the stability studies performed so far demonstrate that rufinamide is a stable and non-hygroscopic substance. Therefore, the applicant's re-test period proposal is justified when the bulk drug substance stored in the proposed packaging material and conditions.

Medicinal Product

- Pharmaceutical Development

At the very early stages of development, uncoated tablets of 1 and 10 mg were produced by direct compression. However, as the dose had to be increased, direct compression was impossible due to the poor flow characteristics of rufinamide. Higher strength (50 and 100 mg) uncoated tablets were developed using the dry "roller" compaction method, but flowability problems were observed during the dry compaction process. As a further increase of the dosage strength to 400 mg was necessary, further changes in the composition and in the manufacturing process of the product were made in order to achieve an acceptable weight and size of the 400 mg tablets and to overcome the technical problems observed in the roller compaction process. A wet granulation method was introduced together with formulation modifications pertaining to partial replacement of water insoluble excipients with water-soluble ones, as well as the employment of stronger disintegrant. In order to improve the wettability and to decrease the broad variability of the bulk density of the drug substance, a pre-densification step was introduced before granulation: the voluminous drug mass is wetted by a solution of sodium laurilsulfate in a high shear mixer. The active substance bulk density specification was set based on results from a bioequivalence study, comparing tablets manufactured with rufinamide of different bulk density. In addition, a particle size specification is proposed to control the quality of the active substance. Other bioequivalence studies were performed between the "roller compaction" formulation and the final formulation (wet granulation with predensification). The final formulation gave 20% higher AUC in bioavailability compared with the "roller compaction" tablets under fed conditions. However, there is no concern from this difference because only the final formulation has been used in the pivotal clinical study.
Apart from the processability problem, another difficulty has been the very low aqueous solubility of the active substance. Therefore, a flow through method was developed to control the dissolution rate of the tablets. Although dissolution times are long this does not present a problem since there is no permeability barrier.
A maximum storage period for the granules was determined to two months. It was further demonstrated that no change of polymorphism occurs during the manufacturing process.
Finally, a hypromellose film coat was introduced to mask the bitter taste of the drug substance. The tablet shape was also changed from round and flat to oblong cores and provision of scores as break-marks.
The tabletting mixture used for the 100, 200 and 400 mg formulations is the same and the different strengths are obtained by increasing proportionally the core weights to avoid different drug release characteristics and to facilitate production.
The excipients are commonly used in pharmaceutical products and were tested for compatibility. All the excipients meet the specifications defined in the current Ph.Eur. monographs or acceptable in-house standards. Purified water is the only solvent used for formulation processes including granulation and coating.

- Adventitious Agents

The magnesium stearate used in the production has been confirmed by the applicant and by the manufacturer to be of vegetable origin and is therefore not considered as a risk regarding BSE.
A certificate from the supplier of the lactose has been provided stating that "the lactose is manufactured from food grade cow's milk, sourced from healthy animals in the same conditions as milk collected for human consumption" fulfilling the NfG CPMP/BWP/1230/98 rev 1, which is satisfactory.

- Manufacture of the Product

Inovelon 100, 200 and 400 mg film-coated tablets are manufactured by standard wet granulation, tabletting and film-coating processes. A common granulate is used for all the three strengths. Granulation and pre-densification are performed on two equal sub-batches. The external phase excipients is added to the combined two sub-batches of granulates, then the mixture is sieved and lubrication is performed in a diffusion mixer.
The manufacturing process involves the following steps: pre-densification, mixing/kneading, wet granulation, drying, sieving/lubrication, compression and film-coating.
Following manufacture, the tablets are packed in bulk into an aluminium laminate bag for transport to the site of packaging into commercial packs.

- Product Specification

The specification for Inovelon film-coated tablets includes tests for appearance (visual), identification (TLC, HPLC), dissolution (Ph.Eur. -Apparatus 4/ HPLC), related substances (HPLC), assay (HPLC), and content uniformity (Ph.Eur.). Additionally, microbiological tests (Ph. Eur) are performed on one batch per year.
The tests and limits of the specifications Inovelon film-coated tablets are appropriate to control the quality of the finished product for the intended purpose.

- Stability of the Product

Eisai has performed stability studies on eight batches of tablets into commercial packaging for twelve months. Results from supporting stability studies performed on drug product stored in HDPE bottles were also presented. Additional stability studies have also been performed on Inovelon tablets in bulk pack. The applicant has committed that the on-going stability studies will continue for three years. The analytical methods used during the stability studies were the same as those applied for control of the drug product.
Stability studies results from twelve batches of all three strengths of Inovelon tablets manufactured by Novartis were presented as supportive data. Eight of those batches were packaged in the intended market packaging material for up to 36 months. Additional stability studies have also been performed on Inovelon tablets packed in HDPE bottles for up to 36 months and in bulk pack for up to 18 months.
Photostability studies have also been carried out on nine batches of tablets of all strengths.
The analytical methods used during the stability studies were the same as those applied for control of the drug product. Additional tests in the shelf-life specification were loss on drying, hardness and disintegration. All results from stressed, accelerated and long-term conditions were well within the specifications limits.

Based upon the on-going stability data from Eisai and stability data from Novartis, the proposed shelf life and storage conditions as stated in the SPC are acceptable.

Discussion on chemical, pharmaceutical and biological aspects

The quality of Inovelon film-coated tablets is adequately established. In general, sufficient chemical and pharmaceutical documentation relating to development, manufacture and control of the drug substance and drug product has been presented. There are no major deviations from EU and ICH requirements. The results of tests carried out indicate satisfactory consistency and uniformity of important product quality characteristics, and these in turn lead to the conclusion that the product should have a satisfactory and uniform performance in the clinic.
Stability tests indicate that the product under ICH guidelines conditions is chemically stable for the proposed shelf life.

3 Non-clinical aspects

The pivotal safety pharmacology studies investigating central nervous system (CNS), cardiovascular system (CVS) (including hERG assay), respiratory and renal function were performed in accordance with Good Laboratory Practice (GLP) regulations.
Most studies and all pivotal studies have been conducted in accordance with GLP, including toxicokinetic analysis.

Pharmacology
The CPMP Note for Guidance (NfG) on treatment of epileptic disorders (CPMP/EWP/566/98 rev1) addresses also non-clinical aspects for a medicinal product to be used for treatment of seizure disorders. "The neurobiological mode of action may be important since it may indicate in which seizure types and epileptic syndrome the drug may by efficacious." Further, "the study of the efficacy profile should be done in several experimental models, including models of generalised epilepsies with absences. It is important to know if the drug in development displays anti-seizure activity only or if it has a potential for antiepileptogenesis as well."

- Primary pharmacodynamics

In vitro studies
Rufinamide *in vitro* (at 1 μM ≈100μmol/L or higher) prolonged the recovery from sustained inactivation of Na^+ channels in cultured cortical neurons investigated with patch clamp technique indicating that rufinamide interacts mainly with the inactivated state of sodium channels and slows conversion to the active state, thereby reducing the frequency of action potentials. No significant inhibition at voltage-gated Na^+, K^+ or Ca^+ channels was observed.
In another experiment, rufinamide dose-dependently decreased the sustained repetitive firing with an IC_{50} of 3.8 μmol/l. The duration of action potential firing also decreased indicating an effect of rufinamide on firing of sodium-dependent action potentials that could contribute to blocking the spread of seizure activity from an epileptogenic focus. No extrapolation between *in vitro* concentrations of rufinamide and the *in vivo* situation in human will be performed since it is considered of limited value.
Rufinamide did not significantly interact with a number of neurotransmitter systems, including GABA, benzodiazepine, monoaminergic and cholinergic binding sites, N-methyl-D-aspartate (NMDA) and other excitatory amino acid binding sites. A weak interaction at β-adrenergic receptor sites was noted at a fairly high concentration.

In vivo studies
The *anticonvulsant activity* of rufinamide was investigated in a large number of relevant animal models of seizure disorders, mimicking generalised tonic-clonic, simple and complex partial seizures. No specific animal model exists for the Lennox-Gastaut syndrome.

In test of generalised tonic-clonic seizures such as the maximal electroshock test (MES test; mice/rats) or on seizures induced by pentylenetetrazol (mice), rufinamide dose-dependently suppressed seizures. In mice an ED_{50} of 19.2 mg/kg was determined using the MES test, and in rats the corresponding ED_{50} is 4-11 mg/kg following oral administration. When extrapolated to pharmacokinetic/toxicokinetic data, these effects were observed at or slightly below clinical exposure. No adverse effects were noted up to 45 mg/kg.
Rufinamide was active also in chemically induced seizures (pentylenetrazole and picrotoxin) in mice at higher ED_{50}'s compared to the MES test, but was without effect in picrotoxin induced seizures. The pharmacological profile is expected to be similar to carbamazepine rather than sodium valproate. The efficacy of rufinamide in the MES test was investigated after different pre-treatment times, varying between 1 and 8 hours. The largest effect was seen after short pre-treatment times, which correlates with the pharmacokinetic profile of rufinamide. When comparing protective indexes for several antiepileptic drugs, rufinamide was equally effective or more effective than conventional antiepileptics.

In animal models of epileptogenesis for partial seizures in cats using penicillin as a focal epileptogen, an inhibition of hippocampal and cortical after-discharges was observed after treatment with rufinamide 300 mg/kg. A reduction of seizures was also observed in chronically epileptic rhesus monkeys with recurring partial seizures after treatment with rufinamide 30-50 mg/kg daily for 15 days.

Evidence from *in vivo* studies using animal seizure models suggests that rufinamide is a long-acting drug.

To assess the effects of rufinamide on learning and memory, the electroshock-induced amnesia test and the step-down passive avoidance test were performed in mice. A reduction in electroshock-induced amnesia and an improvement in learning were observed in each test, respectively.
The analgesic potential of rufinamide was weakly effective in rat and guinea pig models of neuropathic pain (NP). In these models, its overall profile was equal to that of carbamazepine (CBZ), whereas lamotrigine (LTG) was somewhat more efficacious.

- Secondary pharmacodynamics and Safety pharmacology programme

Rufinamide did not produce any unexpected or toxic effects in the safety pharmacology studies, which were conducted in accordance with ICH S7A (CPMP/ICH/539/00).
The main CNS-related effects of rufinamide are slight CNS depressant action in both mice (100-300 mg/kg p.o.) and monkeys (200 mg/kg p.o.) in behavioural tests. In accordance with the proposed mechanism of action the CNS depressant effect is expected.
No effect on motor coordination, body temperature or hexobarbiton induced sleeping time was observed. Further, data on behaviour are also available from primary pharmacodynamic studies indicating few adverse effects except ataxia at high doses. In animal models of learning performance, improved learning was noted in mice at doses of 0.3-30 mg/kg p.o. The clinical relevance of this finding is unclear.

Rufinamide induced no or very slight cardiovascular effects in dogs (up to 10 mg/kg i.v.). No effect on blood pressure, blood flow, P-wave amplitude, P-Q interval, QRS interval and Q-T interval was observed. A slight decrease in heart rate was noted at the low and high dose level. Confirmation of lack of cardiovascular and ECG effects comes from long-term studies in dogs and Cynomolgus monkeys, at exposure level in the clinical range. Rufinamide had no effect on hERG current when investigated in human embryonic kidney cells at concentrations up to 1μM.

Only slight effects on respiratory system, renal system and blood glucose were observed, considered to be of low clinical relevance.

- Pharmacodynamic drug interactions

The ability of rufinamide to interact pharmacodynamically with other antiepileptic drugs was investigated using the MES test. The anticonvulsant effect of rufinamide was additive to that of other antiepileptic drugs. No pharmacodynamic interactions could be observed. Five days repeated treatment with rufinamide induced no tolerance in the MES test, while tolerance developed for diazepam and carbamazepine.

No anticonvulsant activity was observed with any of the rufinamide metabolites CGP 47291, CGP 47292 CGP 47293, CGP 47294 extracted from rat urine.

Pharmacokinetics
The analytical method to determine rufinamide in human plasma was shown to be applicable to dog, rabbit, rat and monkey plasma. Although validation and assays of the toxicology studies samples do not fit to the modern criteria, they were in accordance to the best practice at the time where the studies have been done.

Absorption
Rufinamide was slowly absorbed in all investigated species (mouse, rat, dog, Cynomolgus monkey and baboon), in addition, relatively slow clearance, and little or no first pass metabolism were observed. Non-linear absorption was observed at high doses with decreasing absorption with increasing dose, most markedly in dogs and Cynomolgus monkeys. The low absorption in dogs and baboons could have been caused by the solid dosage form used (crystalline substance in gelatine capsule) whereas for mice, rats and Cynomolgus monkeys, in which absorption was good, a liquid suspension was administered. There were no pronounced gender differences in systemic exposure.

The absorption of rufinamide was characterized by a low rate, with variable extent of absorption which was species-dependant. The absorption capacity for rufinamide was assessed in Caco-2 cells, and the predicted oral absorption was in the order of 80-90%.

Distribution
The distribution was similar in mice and in rats. The highest levels of radioactivity were found in liver (mouse, rat), adrenals (rat) and aorta (mouse). No specific affinity/uptake to organs/tissue was detected and no notable accumulation was observed after repeated dosing. Rufinamide rapidly and reversibly crosses the blood-brain barrier. Rufinamide also crosses the placenta and passes into fetal tissues in rats and rabbits, and was detected also in mammary glands. The serum protein binding was low (23-29%) in rat, dog, baboon and marmoset and comparable to that in man (34%).

Serum protein binding of rufinamide in species was low (23~35%). Radioactivity from labelled rufinamide was distributed throughout the body in rats, and there was no evidence of a peculiar or persistent affinity to specific organs and tissues. A marked and reversible transfer of rufinamide and/or metabolites to the embryo/fetal compartment was observed in rats and rabbits. Radiolabel was distinctly taken up into the mammary glands indicating the compound and/or metabolites could be excreted with the milk.

Metabolism
Biotransformation has been examined in rodents, dogs and primates and the metabolic pathways found include those present in humans. Systemic exposure to metabolites was low. Judging from urinary excretion data, the compound was cleared mainly by metabolism in all species tested and excretion of the products was divided between urine and faeces. Very little unchanged rufinamide was excreted into urine. The main metabolite was the carboxylic acid, designated CGP 47 292, formed by hydrolysis of the carboxylamide group, catalysed by carboxyesterase(s). Oxidative metabolism yielding CGP 47 291 was minor and apparently more pronounced in rodents than in dogs or primates. Rufinamide weakly induced drug-metabolizing enzymes in rat and mouse liver in a qualitatively similar manner to carbamazepine or phenobarbital. Rufinamide showed no significant capacity to inhibit the activity of the human P450 enzymes of relevance to drug metabolism. It can be concluded that the species used in the toxicity studies form the main metabolites in humans (CGP 47 292 and traces of CGP 47 291), and hence, have been exposed to these metabolites.

The choleliths (consisting of metabolite IV) seen in Cynomolgus monkey gall bladder in the repeated-dose studies and the fluoride-linked osteomas in mice observed in the carcinogenicity study, raised questions from the CHMP about their relevance to humans. ,
An additional metabolic pathway (to the metabolism by carboxylesterases) was proposed by the Applicant for the mouse and monkey, where rufinamide is metabolised through oxidation of the difluorophenyl ring, followed by fluoride substitution by glutathione and glutathione degradation via the mercapturic pathway, with subsequent formation of metabolite IV. Thorough investigations by the applicant have shown that glutathione-derived metabolites (including metabolite IV) could not be detected in humans. However, it cannot be completely excluded that small amounts of glutathione conjugates may be formed in humans. Therefore, the CHMP agreed that the effects seen after rufinamide exposure in mouse (osteomas and degeneration of submandibular glands) and Cynomolgus monkey (choleliths in gall bladder consisting of metabolite IV) can be considered as species specific.

Excretion
Excretion of rufinamide was rapid and complete in mice, rats, dogs, monkeys and baboons. Renal excretion was the predominant route of excretion in all species, except the baboon in which biliary excretion appeared to be significant.

Toxicology
All pivotal studies were conducted according to GLP standards.

- Single dose toxicity

Rufinamide is of low acute toxicity with approximate lethal oral doses of more than 5000 mg/kg in mice, 5000 mg/kg in rat and more than 2000 mg/kg in dogs. With intraperitoneal injection in rats the lethal dose was 1000 mg/kg. The clinical observations were mainly CNS related. No toxicokinetic data were available from the single toxicity studies. When comparing allometrically corrected non-lethal doses, the multiple to human maximum dose (48 mg/kg) was approximately 10 in the mouse and rat, and 20 in the dog.

- Repeat dose toxicity (with toxicokinetics)

Rufinamide was administered orally to mouse (3 months and 600 mg/kg/day), rat (up to 26/52 weeks and 600 mg/kg/day), Beagle dog (up to 26/52 weeks and 600 mg/kg/day), Cynomolgus monkey (12 months and 300 mg/kg/day) and wild-caught baboon (1 month and 300 mg/kg/day). The main target organ of toxicity was the liver in all species tested, and in rodents, the kidney.

In mice, up to 3 times increases in AST/ALT and ALP and hepatocellular hypertrophy (minimal to mild in males and minimal in females), single cell necrosis and/or hepatic pigment accumulation (in periportal areas, in Kupffer's cells and hepatocytes) accompanied by increased relative and absolute liver weights at 200 and 600 mg/kg were seen. These effects occurred at an exposure level similar to the maximum clinical exposure (AUC). Although hepatocellular hypertrophy and increased liver weights are indicators of metabolic induction. rufinamide was only a weak inducer of drug-metabolising enzymes in male mice. The accumulation of unidentified pigment and increased ALT/AST and ALP is indicative of hepatic injury. Liver was also a target organ in the repeated-dose toxicity studies in rat and in the carcinogenicity studies in rat and mouse. The mechanism of these findings is not fully explained and the relevance for humans is uncertain. However, no significant liver toxicity were observed during the clinical trials.

Atrophy of the acinar epithelium of the submandibular salivary glands was characterised by a decreased number of secretory granules and increased amounts of pale, basophilic cytoplasm, occasionally vacuolated. These changes were of minimal severity, with the exception of one high dose female, which showed gland degeneration. The metabolic pathway in the mouse liberates fluoride ions (see pharmacokinetics) and a correlation between fluoride and the development of the submandibular glands has been discussed in the literature. As the fluoride liberating pathway is not relevant to humans, (see above section metabolism), the clinical relevance of the effects on the submandibular glands are not considered to be an issue for human safety.

In rats, mostly in male and to a much less extent in females, administration of rufinamide for 1 month and up to 52 weeks caused centrilobular hepatocellular hypertrophy and cytoplasmic vacuolation of cells of the anterior pituitary. These findings are related to increased T4 UDP-GT activity, seen after 8 days with a dose of 600 mg/kg. T4 UDP-GT enhances the clearance of thyroid hormones, which results in a reduction of negative feedback, activating TSH in the pituitary, and, in turn, activating the thyroid to produce thyroid hormones. Liver enzyme induction leading to disruption of the pituitary-thyroid axis is well known and species specific for the rat, and thus lacks relevance to human risk assessment. Hepatocyte enlargement and pigment accumulation in macrophages and Kupffer's cells were seen at mid and high dose, even after 4 weeks recovery

In Beagle dogs, two moribund females were seen at mid and high doses in the 13-week study (87-6091) (another female at high dose had the same symptoms, but developed them later and could continue the study). The severe signs, clinical collapse, severe haematology and bone marrow changes, could be attributed, according to the applicant, to an auto-immune reaction.

Further, at doses of 600 mg/kg, hepatic effects included periacinar, intracanalicular or centrilobular intrahepatocellular bile accumulation, bile plugs and pigment deposits within hepatocytes or in bile canaliculi and focal perivascular inflammation with increased ALP, AST and ALT. Only partial reversibility was seen after 4 weeks in the 26/52 week study. The AUC for the 600 mg/kg dose was (mean female and male) 4430 µmol*hr/L, giving an exposure multiple to the maximum clinical exposure of approximately 2. Clinically, a 5 time increase of the above upper limit of normal was seen for bilirubin, AST and ALT in a few patients. Since the metabolic pathways in dogs and humans are similar, and metabolite CGF 47292 being a common major metabolite, the CHMP requested discussion on this finding. Investigations on the bile physiology in bile-duct cannulated dog that repeat-doses of rufinamide induced a change in bile composition and viscosity as well as an hypercholeresis. Although the mechanism of the cholestasis is not understood, the clinical experience overrules the question of the relevance to humans of these findings. In the dog, the NOAEL is set to 5 mg/kg (13-week study), where leukocytic infiltrates in the liver was seen in female dogs.

In non-human primates (Cynomolgus and baboon monkeys), the major findings were reversible liver weight increases, minimal transaminase increases, reversible hepatocellular hypertrophy and formation of choleliths in the gallbladder lumen. These choleliths were seen at necropsy in the 13-week study in both male and female monkeys in more than half of the mid-dose and in all monkeys in the high-dose, and in the 52-week toxicity study in 2 out of 7 monkeys at termination. In the 13-week study, these crystals were even seen in one high dose female after 4 weeks of recovery. No choleliths were found in the 1-month exploratory study in baboons. Analysis by HPLC with UV diode array detection, LC-MS and ^{1}H-NMR spectroscopy, revealed that the choleliths consisted of Metabolite IV, the insoluble 3-hydroxy-6-fluoro-2-S-cysteinyl conjugate of rufinamide. Metabolite IV is formed by fluoride substitution by glutathione and subsequent glutathione degradation via the mercapturic pathway. In the 52-week toxicity study, metabolite IV was present in urine and bile after 26 weeks of exposure to rufinamide, and at all dose levels, but absent after 4 weeks recovery. Metabolite IV has also been detected in rat and in dog bile. As explained above, as metabolite IV is not found in humans these findings are considered not to represent a risk for humans.
In the Cynomolgus monkeys, the NOAEL was set to 20 mg/kg, and in the baboon, the NOAEL was set to 30 mg/kg in the 1 month study. These exposures corresponds to approximately half the human exposure.

- Genotoxicity

Rufinamide has been studied with respect to gene mutations in bacteria and mammalian cells and chromosomal aberrations *in vitro* and *in vivo*. Additional tests of nucleus anomality and sister chromatid exchange have been conducted *in vivo* in Chinese hamsters. In all three *in vivo* tests, a maximum dose of 5000 mg/kg by oral gavage was used, and the animals are considered adequately exposed. No genotoxic potential was evident at concentrations up to the limit of toxicity or where signs of toxicity were observed.

- Carcinogenicity

104-week dietary carcinogenicity studies have been performed I the mouse and rat.
In mice, rufinamide was administered in the diet at doses of 0, 40, 120, or 400 mg/kg. Body weight gain reductions were-17% in males and -18% in females at 400 mg/kg. At 200 and 600 mg/kg, effects of rufinamide treatment were seen in the liver, bone and urinary system.

In the bone, hyperostosis was recorded, which is known to result from chronic, increased fluoride exposure. Treatment-related myelofibrosis was seen at mid and high dose in both females and males, even though no dose-response was seen for the females. The number of mice affected was 0, 1, 4, 10 and 3, 4, 10, 6 at 0, 40, 120 and 400 mg/kg, for males and females, respectively.

In the bone, osteomas were seen. Osteomas are benign and slowly growing neoplasms and most of them originate from the osteoblastic layer of the periosteum. Spontaneous osteomas appear at low frequency in most strains of mice, but these tumours can also be induced by mouse-specific polyomavirus and retrovirus. They are usually located to skull and larger bones of the limbs. In the cancer study in mouse, 0, 2, 3 and 9 mice with osteomas with periosteal origin were seen at 0, 40, 120 and 400 mg/kg, respectively. Multiple osteomas were observed in 7/14 osteoma-bearing mice and the most common locations of the osteomas were skull and pelvis. Thus, the osteomas displayed typical

characteristics of virus-induced osteomas. Additional electron microscopic analyses (included in the cancer study, but not conducted according to GLP) were performed on bone specimens from the mouse cancer study. Out of 11 examined samples, 5 did show occasional particles of appropriate size, but no evidence of budding or viral substructure. The remaining samples did show intracytoplasmic particles with c-type retroviral morphology, size and budding in association with osteoblasts, osteocytes and endothelial cells. The low incidence of osteomas in controls of contemporary studies, and no osteomas in the controls of the rufinamide cancer study makes it likely that the osteomas seen have mixed mechanistical origin, where both the presence of mouse-specific retroviruses and high levels of fluoride contribute to the development of osteomas. Neither of these contributors are relevant to human risk assessment, considering that no fluoride substitution takes place in the human metabolic pathway for rufinamide (see above). In the toxicokinetic study, the AUC is estimated to be 2400 µmol·hr/L at 400 mg/kg.

The changes in the urinary system consisted of minimal hydronephrosis, focal fibrosis in the kidney, dilation of the ureters and bladder affected mainly males in the high dose, likely due to high age.

In the liver, the same effects as in the pivotal repeated-dose toxicity studies were seen; increases in AST/ALT and ALP, liver hypertrophy and pigment accumulation in the macrophages (see above).
In addition, treatment-related increases in the incidences of hepatocellular adenomas and carcinomas were observed in both sexes at 400 mg/kg. The mouse liver is known to be sensitive to tumourigenisis from agents causing microsomal enzyme induction, however, rufinamide was only a weak inducer of drug-metabolising enzymes in male mice. Comparison with historical controls for this strain shows that the incidence of liver tumour is within the range of the biological variability of the CD-1 strain of mice. No liver neoplasms were seen in the rat carcinogenicity study. Therefore this finding is not considered to be a concern for humans.

In rats, rufinamide was administered in the diet at doses of 0, 20, 60, or 200 mg/kg. Polyuria in both sexes at the high dose, and reduced body weight and food consumption were observed at all dose levels, with reductions in body weight gain of -30% in females and -13% in males at the high dose at termination. Plasma concentrations of rufinamide were detected in 6 out of 89 control samples, at approximately 4 times LoQ (0.05 µg/ml). This is not considered to invalidate the study.

In the liver at 60 and 200 mg/kg, centrilobular hepatocellular hypertrophy and megalocytosis was seen. It is agreed that centrilobular hepatocellular hypertrophy is related to enzymatic induction seen in rats. Megalocytosis might also be correlated with hepatocellular hypertrophy. However, megalocytosis occurred primarily in the females with 9/60 rats affected in the high dose, while 14/60 rats had centrilobular hypertrophy. In the males no cases of megalocytosis was seen in any dose group, but 16/60 and 36/60 rats were diagnosed with centrilobular hypertrophy in the mid and high dose, respectively. In addition, accumulation of pigmented hepatic macrophages and apoptosis, seen only in high dose females with 4/60 rats affected, were observed. As mention earlier (see repeat-dose toxicity), although the mechanism of liver findings is not fully understood, no significant signals of hepatoxicity have been identified in the clinical trials.

In the kidney, pelvic epithelial hyperplasia and pelvic mineralization was observed in both sexes at 60 and 200 mg/kg. Toxicity to the kidney (tubular dilation in the renal medulla and mineralization) at 60 mg/kg was seen in the dose-range finding study in juvenile rats (dosed up to day 35 post partum), and in the repeated-dose toxicity study 92-100. As kidney lesions were not found in longer repeat-dose toxicity studies and as these findings could be attributed in the carcinogenicity study to spontaneous lesions in aged rats (chronic progressive nephropathy), it was concluded that they were not considered of toxicological significance.
In addition, decreased bone marrow cellularity in males at 60 and 200 mg/kg, thymic atrophy in females at all doses and ovarian stromal hyperplasia at 60 and 200 mg/kg was seen. Ovarian stromal hyperplasia is likely related to old age. Since no effect was seen on the ovary in other toxicity studies, this is not considered to be a concern.