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Pregabalin (Lyrica)

2004/9570

Application No: 04/111/

Pfizer

Australian Government
Department of Health and Ageing
Therapeutic Goods Administration

NONCLINICAL EVALUATION OF REGISTRATION APPLICATION

NEW CHEMICAL ENTITY

Sponsor:	**Pfizer Australia Pty Ltd**
Generic name:	**Pregabalin**
Trade name:	**LYRICA™**
Drug class:	**Analgesic, Anti-epileptic**
Dose form and strengths:	**25 mg, 50 mg, 75 mg, 100 mg, 150 mg, 200 mg, 225 mg, 300 mg capsules**
Submission No:	**2004-0021-1**
Toxicology file No:	**2004/009570**
Evaluator:	s22 [redacted] s22 [redacted]
Date:	**29 October 2004**

Note: This evaluation has been checked for confidential information and is cleared for release to the Sponsor only after this cover page and page 8 are replaced with the attached edited pages.

SUMMARY

- Pregabalin is an analogue of γ-aminobutyric acid (GABA) related to the anticonvulsant gabapentin. It is intended for the treatment of neuropathic pain and partial seizures, with an oral dose of 150 mg/day increasing to 600 mg/day. It was shown to penetrate the blood brain barrier, at least to some extent, with brain/plasma ratios of s47 (rats) or *ca* s47 (cynomolgus monkeys) after continuous IV infusion. Efficacy was adequately demonstrated in appropriate rodent models, with the R-enantiomer being essentially inactive, and although the exact mode of action is not clear, pregabalin, like gabapentin, bound to the $\alpha_2\delta$ subunits of voltage-gated Ca^{2+} channels *in vitro*. Binding to rat brain $GABA_A$ or $GABA_B$ receptors was not seen, and pregabalin had no effect on GABA transaminase *in vitro*. There were no safety pharmacology concerns, including potential for QTc interval prolongation, and potential for abuse or dependence appeared to be limited. Overall, there were no outstanding issues and the nonclinical dossier was sufficient to support this application for registration, despite an oncogenic response to long-term treatment in mice (below).

- As in humans, pregabalin metabolism was minimal in the species used in the toxicity studies, mice, rats and cynomolgus monkeys, but extensive conversion to the N-methyl derivative occurred in dogs. Pregabalin had no effect on reactions catalysed by common human microsomal CYP isoforms *in vitro*. Plasma drug clearances after s47 mg/kg IV administration were s47 (mice), s47 rats) and s47 (cynomolgus monkeys) mL/h/kg, and as in humans, excretion was overwhelmingly urinary. Pregabalin did not bind to rat, cynomolgus monkey or human plasma proteins *in vitro*, and conversion to the R-enantiomer was not measurable in selected toxicity study plasma samples. Oral bioavailability was high in mice, rats and cynomolgus monkeys (76-94% with s47 /kg given by gavage), with an estimated human value of >90% based on urinary excretion data. Dietary or gavage administration was used in the toxicity studies.

- Drug animal/human exposure ratios (ER, based on plasma AUC) were sufficient to define the toxicity profile of pregabalin, although values in the pivotal cynomolgus monkey study were not high (up to *ca* 8). Haematological and bone marrow changes in mice were apparently related to haemangiosarcoma formation (below), and were extensively investigated in a series of supplementary studies. In contrast to mice, in which elevations occurred, platelets were decreased in rats or unaffected by treatment in cynomolgus monkeys. Other salient findings in the toxicity studies included tail lesions (erythema, dermatopathy, necrosis), which generally resolved with continuing treatment, testicular tubular degeneration and retinal atrophy. The latter, seen only in rats, appeared to represent exacerbation of an age-related change. CNS-related clinical signs (ataxia, hypoactivity) seen with higher doses in shorter-term studies were considered to reflect exaggerated pharmacological activity. There was no unexpected toxicity in a 7 week study using young rats, and fertility was unaffected, with doses achieving drug ER values of up to 11. Abnormalities of acoustic startle responses, of unknown significance, were observed in young rat behavioural neurotoxicity studies.

- Pregabalin was not genotoxic in adequate assays, but increased incidences of haemangiosarcomas were seen in B6C3F1 mice, and to a lesser extent in CD-1 mice, but not in rats. A significant effect was seen at s47 mg/kg/day in the more sensitive strain, associated with expected drug ER values of *ca* 6-33, compared with *ca* 1 at the NOEL of s47 mg/kg/day. There were no other significant treatment-related oncogenic findings in either species. Extensive mechanistic studies showed a strong association between platelet activation, megakaryopoiesis, liver endothelial cell proliferation and haemangiosarcoma formation. Because of its apparent epigenetic origin, and differences in rat and cynomolgus

monkey platelet and bone marrow responses to treatment, compared with mice, this oncogenic finding is probably of limited relevance to humans (see *Assessment: Genotoxicity and carcinogenicity*).

- Embryofetal development studies did not show any treatment-related teratogenicity in mice, rats or rabbits at doses achieving drug ER values of up to s47 Premature closure of the zygomaticomaxillaris and nasofrontalis sutures was seen in rats at high doses, with a high drug ER value (*ca* 17) at the combined NOEL of s47 mg/kg/day (low-dose). These skull findings were initially classified as malformations but subsequent ancillary studies showed that they were normal stages in fetal skull development. For overall embryofetal toxicity, this was not seen in mice or in the absence of maternal toxicity in rabbits, but a NOEL could not be determined in rats, although there were only minor skeletal variations at s47 mg/kg/day. No male-mediated embryofetal toxicity was seen in rats.

- Male rat fertility was reversibly impaired at high doses, associated with markedly decreased sperm quantity and quality, but the expected drug ER value was high (*ca* 11) at the NOEL of s47 mg/kg/day. No adverse effects of treatment were seen on semen values in the pivotal cynomolgus monkey toxicity study. Female rat fertility (pregnancies/mating) was unaffected by treatment, but increased post-implantation losses were evident, with an overall NOEL of s47 mg/kg/day (expected drug ER value of s47 Several adverse effects of treatment were seen in a rat pre- and post-natal study, including interference with parturition and increased pup mortality, but expected drug exposures with the higher doses used were excessive. An overall NOEL of 50 mg/kg/day was obtained for developmental toxicity, with an associated drug ER value of *ca* 2. Substantial concentrations of pregabalin were measurable in rat milk.

- There are no objections on nonclinical grounds to the registration of pregabalin as proposed.

ASSESSMENT

The submitted dossier was comprehensive and of adequate quality, although some of the GLP studies were not signed by the study director, as indicated. Others were either electronically signed or were stamped 'signatures on file'.

Efficacy. The efficacy of pregabalin has been adequately demonstrated in models of neuropathic pain and epilepsy. The former included rats with sciatic nerve constriction and streptozocin-induced diabetic neuropathy, and it was noteworthy that allodynia was decreased in both models after intrathecal as well as PO administration, suggesting a central site of action. Dorsal root reflexes or reflex motor responses to pinch stimuli in these 2 models were shown to be depressed by IV treatment. Pregabalin also showed analgesic activity against nociceptive pain elicited by several treatments including formalin, carrageenan and acid saline injections, UV irradiation and surgical incision in rats, and hot water in rhesus monkeys, and where tested, the R-enantiomer showed little or no activity. Activity against seizures elicited by several treatments (maximal electroshock, pentetrazol, audiogenic stimulation, electrical hippocampal stimulation) was demonstrated, and again the R-enantiomer was essentially inactive, but pregabalin had no activity in a rat strain exhibiting spontaneous seizures.

Mode of action. Despite extensive investigations the exact mode of action of pregabalin is not clear. However, the registered anticonvulsant, gabapentin, has been shown to bind to the $\alpha_2\delta$ subunits of recombinant voltage-gated Ca^{2+} channels (porcine type 1 and to a lesser extent human type 2), and pregabalin also bound to these proteins as shown by displacement of bound [^{3}H]gabapentin, suggesting common receptors for both drugs. Binding to this protein is implicated in the mode of action of pregabalin as shown by *in vivo* inactivity of the R-enantiomer

(analgesic and anti-convulsant), which shows a >10-fold lower *in vitro* binding to $\alpha_2\delta$ subunits 1 and 2 (based on displacement K_i values), and tests using a transgenic mouse strain containing a single amino acid substitution in the $\alpha_2\delta$-1 subunit. This resulted in decreased *in vitro* [^{3}H]gabapentin binding to brain membranes (wild type>heterozygous>homozygous), while PO (100 mg/kg) pregabalin showed analgesic and anxiolytic activity in wild type and heterozygous mice but not in homozygous mice. However, pregabalin ED_{50} values for protection against maximal electroshock seizures were not greatly affected (7.8 (wild type), 24.7 (heterozygous) and 19.7 (homozygous) mg/kg), suggesting that other mechanisms may be operative in anticonvulsant activity, at least in this model.

Secondary pharmacodynamics/safety pharmacology. Investigations of the safety pharmacology of pregabalin were variable, but generally adequate. Little or no cardiovascular response was observed in rats (PO, IV), dogs (PO) or cynomolgus monkeys (IV), while measurement of quantitative ECG values in the pivotal cynomolgus monkey toxicity study showed no effects of treatment at doses achieving animal/human systemic drug exposure ratios (ER) of up to s47 (below). Pulmonary function was measured in anaesthetised dogs after s47 mg/kg IV 50 min infusion, but although there were said to have been no effects of treatment, data were not included in the study report. It is noteworthy that respiratory values were measured in short-term (AA3012) and long-term (AA2787) supplementary studies in female B6C3F1 mice, with respiratory depression (which probably reflected CNS activity) being observed at respective dietary doses of s47 mg/kg/day. Expected drug ER values with these doses were high (respectively *ca* 32 and 7-8, see table below), but lower doses were not tested and the relevance of these findings to humans was not clear, although the apparent lack of respiratory responses in dogs suggests that it may be minimal. Respiratory rates were unaffected by treatment in the clinical trials (clinical overview). The observed respiratory depression in mice may indirectly alter megakaryopoiesis and platelet function, factors thought to be involved in haemangiosarcoma formation in this species (see *Toxicity: Genotoxicity and carcinogenicity*).

Urinary volume was increased in rats treated IV with s47 mg/kg IV, although this was associated with increased water consumption, and total 24 h electrolyte excretions were unaffected by both doses. However, gastric emptying and intestinal transit of radiolabel were retarded by PO treatment in rats, and constipation is noted in the proposed product information as a common effect of treatment. As may be expected for a CNS active drug such as pregabalin, high doses elicited a number of clinical signs, including ataxia, hypoactivity, decreased body temperature, hypnotic-like sleeping patterns, impaired righting reflexes and motor co-ordination. Dizziness, abnormal co-ordination and ataxia were noted as very common or common effects of treatment in the proposed product information.

Secondary pharmacodynamic findings included a lack of *in vitro* binding to $GABA_A$ and $GABA_B$ receptors, and demonstrated anxiolytic, anti-inflammatory and anti-arthritic activities, and protection against indomethacin-induced gastric lesions. Pregabalin was also active in various models of colonic pain.

Dependence. Pregabalin showed little potential for abuse or dependence, based on behavioural studies in rats and rhesus monkeys, and although some withdrawal signs were seen in rats after 12 days of continuous IP infusion, these were less than with pentobarbitone. Additionally, *in vitro* binding to human recombinant cannabinoid receptors, as well as animal-derived benzodiazepine and opioid receptors, was not demonstrable.

Pharmacokinetics and relative drug exposures. Plasma pregabalin exposures achieved in the PO pivotal toxicity and carcinogenicity studies are summarised in the following table.

Species	Doses (mg/kg/day)*, study duration (sample week)	$AUC_{0\text{-}24\ h}$ (µg.h/mL)	Exposure ratios (ER)@
Mouse (B6C3F1)	s47 [redacted]	s47 [redacted]	s47 [redacted]
Mouse (B6C3F1)			
Mouse (CD-1)			
Rat			
Rat			
Cynomolgus monkey			

* dietary (rodents) or gavage (cynomolgus monkeys) administration
@ compared with a human plasma $AUC_{0\text{-}24\ h}$ value of s47 [redacted] µg.h/mL (**1008-002**, section 6.8)
& supplementary 4 week toxicokinetic studies
# AUC values not available for this study, values from a 4 week toxicity study
** this dose given for only 13 weeks
nd = no data

Achieved ER values were sufficient to define the toxicity profile of pregabalin, and although they were not high in cynomolgus monkeys, the high-dose elicited marked tail lesions in the 13 week interim phase of the pivotal study. PO gavage doses used in the embryofetal development studies were high and achieved ER values of s47 [redacted] (mouse), s47 [redacted] (rat) and s47 [redacted] (rabbit), while ER values of s47 [redacted] were achieved with PO gavage doses of s47 [redacted] mg/kg/day in 2 pre- and post-natal studies. Higher doses tested in one of the latter studies elicited marked maternal toxicity and there were consequently only limited toxicokinetic data. Two fertility studies were carried out in adult male rats, with a lower dose range of s47 [redacted] mg/kg/day in the second study (to determine a NOEL) resulting in ER values of s47 [redacted] Only 4 h plasma drug concentrations were measured in the female rat fertility and early embryonic development study, but ER values with the doses used s47 [redacted] mg/kg/day) would have been high (i.e. 17-80), based on data from the embryofetal development study.

Steady state pregabalin brain/plasma ratios were s47 [redacted] (rat) or *ca* 0.1 (cynomolgus monkey) in toxicity studies with continuous IV infusions, showing limited brain penetration. A similar rat (and male mouse) value was obtained for the brain/blood radioactivity ratio at the time of peak concentrations after PO [^{14}C]pregabalin administration in a tissue distribution study (quantitative whole body autoradiography), while a higher value (*ca* s47 [redacted] was obtained at 4 h in cynomolgus monkeys.

Plasma metabolites. Plasma radioactivity was shown to be virtually all associated with unchanged drug in mice, rats and cynomolgus monkeys after PO administration of [^{14}C]pregabalin. Although there were apparently no corresponding human plasma data, metabolism in humans was minimal, as shown by urinary radioactivity recoveries. Excretion in humans was overwhelmingly urinary (90% of the dose vs 0.1% in the faeces), with only traces of the N-methyl derivative and an unknown metabolite being present. A similar predominance of urinary excretion was seen in mice, rats and cynomolgus monkeys, but with a slightly higher faecal excretion, and only minor metabolites (including the N-methyl derivative) were present. The dog showed a different pattern, with extensive metabolism to the N-methyl derivative (44.6% of urinary radioactivity) and this species was not used for toxicity studies.

Pregabalin is the S-enantiomer and the possibility of enantiomeric conversion in experimental species was addressed, with no R-enantiomer being detected in plasma samples from mouse, rat,

rabbit and cynomolgus monkey toxicity studies. There was apparently no corresponding investigation for human plasma.

Toxicity. Besides increases in haemangiosarcomas in mice (see *Genotoxicity and carcinogenicity*) several toxicities were identified, with some findings being relevant to the possible pathogenesis of this apparently epigenetic oncogenic response. Salient findings in the pivotal (including carcinogenicity) studies are tabulated below.

Findings	Mouse (B6C3F1)	Mouse (CD-1)	Rat	Cyno. monkey
Platelet number	↑	↑	↓	
Platelet volume	↑	↑	↑	
Erythrocytes			↑	
Blood smear abnormal platelets/schistocytes*	+	+		
Bone marrow cellularity (histology)	↑		↓	
Bone marrow ↑ megakaryocytes*	+	+		
Tail dermatopathy			+	+
Retinal atrophy			+	
Testicular tubular degeneration	+		+	(+)#
Lung alveolar macrophages	+*	+	+	
Uterus dilation			+	
Urinary staining/bladder dilation/	+	+	+	

* retrospective finding # hypospermia seen in a 4 week study (**1929**)

In contrast to mice, platelet numbers were consistently decreased in rats or unaffected in cynomolgus monkeys, and because of the putative role of platelet function in mouse haemangiosarcoma formation (below), effects of treatment on platelets and bone marrow were extensively investigated in a series of supplementary studies. Mild-moderate (by 4-36%) thrombocytopenia was seen with all doses in the rat pivotal toxicity and the 2 carcinogenicity studies. However, the lack of similar findings in cynomolgus monkeys with drug ER values of up to 9 in the pivotal study suggested that this may not be applicable to humans, and no clinically significant changes in platelet numbers were apparent in the clinical trials, with doses of up to 600 mg/day for up to 36 months (clinical overview).

Rat thrombocytopenia was associated with bone marrow hypocellularity and quantitatively decreased numbers of nucleated cells in the pivotal toxicity study (MD, HD), while a slightly decreased number of megakaryocytes was measured in HD group rats of one carcinogenicity study. No effects of treatment on myelogram values were apparent in the pivotal cynomolgus monkey study, other than a slightly lower number of megakaryocytes in the HD group, as in rats. Increased megakaryocytes (quantified per 5000 haematopoietic cells) were seen with all doses in both mouse carcinogenicity studies, in retrospective examinations, as may be expected considering the elicited thrombocytosis.

Retrospective examination of blood smears from the mouse carcinogenicity studies showed platelet abnormalities (non-uniform size, aggregates, evidence of degranulation), considered to be surrogate markers of activation, and the presence of schistocytes (fragmented erythrocytes). These were not present in the cynomolgus monkey pivotal study, or in the human clinical trials, further supporting the different response to treatment in mice, although this was not always apparent (e.g. in CD-1 mice treated with s47 [redacted] ng/kg/day x 12 months, AA2892). Blood smear examination in the pivotal rat study showed increased large/giant platelets in the mid- and high-dose groups but details were sparse and there was no mention of aggregates or degranulation, while a low incidence of schistocytes was apparent in all groups. The nonclinical expert used results from a supplementary 12 month toxicity study in female rats (AA2796) to support the lack

of abnormal platelet morphology, but this was apparently based on ultrastructural observations at 2 months rather than blood smears and so was not directly comparable.

Ex vivo platelet aggregation was specifically measured in the pivotal cynomolgus monkey study, with no effect of treatment on ADP-induced aggregation, which contrasted with decreases seen as early as 4 weeks in supplementary mouse studies (e.g. AA2795, 2935). Mouse platelet aggregation was characterised by dissociation of initial aggregates resulting in a lower terminal aggregation, an effect not seen in rats (study AA2796) or in human clinical trials (clinical overview). Overall, although data were sometimes variable, mouse platelet and bone marrow responses to treatment appeared to be markedly different from those in rats and cynomolgus monkeys.

There were indications that the testes were a target organ in rodents, with variable findings of reduced testicular weights, tubular epithelial degeneration/atrophy, hypospermia/aspermia or epididymal luminal debris, although these were not present in the pivotal cynomolgus study in which semen analysis did not show any effect of treatment. The lack of such findings in primates at an adequate drug ER value (hypospermia noted in a 4 week study may have reflected animal immaturity) suggests that this may not be the case in humans, and semen analysis in a 3 month clinical trial did not show any effects of treatment (clinical overview). Male rat fertility was decreased by treatment, associated with sperm and testicular/epididymidal changes (see *Reproductive toxicity*).

Tail lesions, including erythema, dermatopathy and necrosis, occurred in rats and to a greater extent in cynomolgus monkeys, although in the pivotal studies these were transient (over 1-4 weeks in rats) or had generally resolved by the study end (cynomolgus monkeys). Histological correlates (epidermal ulceration, dermal neutrophilic inflammation) were, however, present in cynomolgus monkeys at the 13 week interim kill. Additional findings after continuous IV administration were dermatopathy not restricted to the tail, widespread clinical swelling and histological findings of dermal/SC vasculopathy, vascular necrosis associated with epidermal degeneration/ulceration and widespread SC oedema. Review of skin sections from this study by 2 external consultants suggested that primary changes were endothelial cell damage and thrombosis, with secondary epidermal/dermal degeneration and necrosis resulting from vascular damage (745-02999). Although this is not the intended treatment protocol, and doses used achieved continuous concentrations that were s47 the human C_{max} value of 9.1 µg/mL, findings may be relevant to a slightly increased incidence of peripheral oedema noted in treated patients (clinical overview).

Increased retinal atrophy was seen only in the 2 rat carcinogenicity studies, with mainly females (AA2235) or both sexes (AA2299) being affected, and the latter also showed increased mid- and high-dose male incidences of chronic corneal inflammation, and keratitis with neovascularisation at ophthalmic examination. For the combined studies, the NOEL values for retinal findings were s47 ng/kg/day (low-dose, males) or s47 mg/kg/day (low-dose, females). A re-examination of sections from study AA2235 by an external consultant confirmed this retinal finding, and it was suggested that it represented an exacerbation of an age-related change (745-03298). This was supported by the lack of retinal changes in control and high-dose rats in the 52 week pivotal study (interim and terminal kills), while there were low incidences of this finding in control females from both carcinogenicity studies. Retinal findings were also not a feature of the pivotal cynomolgus monkey study, at either the original histological examination or after re-examination by an external consultant (745-03852).

Other findings of unknown significance included urine staining, a prominent clinical sign in rodents sometimes with associated findings of bladder dilation. There were also some bladder

histological findings, but marked lesions (haemorrhagic mucosal necrosis, epithelial hyperplasia) occurred only in premature deaths in short term studies with high doses. Only lamina propria haemorrhages (minimal-moderate) were seen in the rat pivotal study (high-dose males affected), while no bladder lesions were apparent in the mouse or rat carcinogenicity studies. Increased uterine weights associated with gross and histological distension were seen only in the 2 rat carcinogenicity studies, with a clear effect at the high-dose (ER value of *ca* 24), while increased lung alveolar macrophages in rodents possibly reflected phospholipidosis. There were indications of cardiotoxicity in an initial 4 week study in cynomolgus monkeys, with findings of increased heart weights, heart enlargement/ventricular hypertrophy and histological degeneration/necrosis/fibrosis. This was thoroughly investigated in the subsequent pivotal study, which included additional ECG, echocardiography and blood pressure measurement, with no myocardial findings.

Tissue distribution studies showed high pancreatic concentrations of radioactivity after PO administration of [^{14}C]pregabalin to mice and rats, but not cynomolgus monkeys. However, this was not associated with specific toxicity in this organ, although there were some minor sporadic findings in rats (e.g. decreased acinar granules). [A similar pancreatic distribution in rodents was reported for gabapentin.]

Genotoxicity and carcinogenicity. No pregabalin genotoxicity was apparent in adequate assays, which included *ex vivo* unscheduled DNA synthesis and *in vivo* micronucleus formation in the 2 strains of mice used in the carcinogenicity studies. PO gavage doses were up to s47 mg/kg, also used in the corresponding rat tests, which would have been expected to have achieved high plasma concentrations s47 based on comparison of C_{max} or 4 h values in the reproductive toxicity studies with a human C_{max} value of 9.1 µg/mL). *In vitro* assay concentrations were to s47 µg/plate (bacterial reverse mutation) or s47 µg/mL (mammalian cell gene mutation, chromosomal aberrations), which is equivalent to *ca* s47 nM.

However, increases in haemangiosarcoma incidences were seen in B6C3F1 mice used in an original study, and to a lesser extent in CD-1 mice used in a confirmatory study conducted at a different laboratory, with both sexes being affected. Significant increases were apparent with doses expected to achieve ER values of *ca* 6-33 (B6C3F1, mid- and high-dose) and *ca* 28 (CD-1, high-dose), but a similar responses was not observed in 2 Wistar rat studies in which expected ER values were up to *ca* 25 in females. In a further study (AA2787), it was shown that increases in female B6C3F1 mice haemangiosarcoma incidences occurred after s47 mg/kg/day treatment for 104 weeks or for 52 weeks followed by a 52 week recovery period. Although clear increases were demonstrated, this tumour was seen in control mice at incidences of 3-8.3% (B6C3F1, both sexes in the main study and females in 2 arms of study AA2787) and 3-9.2% (CD-1, male and female). There were no other treatment-related oncogenic findings.

The lack of demonstrated genotoxicity is suggestive of an epigenetic mechanism for this tumour, which arises from vascular endothelial cells and which was most prominent in the liver, spleen, bone marrow and uterus (CD-1 only). Endothelial cell and platelet functions are known to be intimately linked and as noted above, associated changes in the mouse carcinogenicity studies included increased platelet numbers and volume, and in B6C3F1 mice, increased bone marrow hypercellularity. Morphological abnormalities of platelets and the presence of schistocytes were also seen, with the latter probably reflecting changes in shear stress related to endothelial abnormalities.

Subsequently a number of *in vitro*/*ex vivo* and *in vivo* mechanistic studies were carried out in which there was little or no evidence for pregabalin acting as an endothelial cell mitogen, altering platelet function or stimulating megakaryocyte development *in vitro*. Different treatment

protocols, the large number of values measured and natural variation in the *in vivo* studies (mainly using B6C3F1 mice), made inter-study comparisons sometimes difficult. However, *inter alia*, treatment did not affect mouse platelet half-lives, but increases in liver endothelial cell proliferation (variable), platelet numbers and volume, bone marrow megakaryocytes and platelet activation (P-selectin expression) were generally observed, together with platelet changes (decreased/abnormal aggregation, platelet morphological abnormalities).

Some increases in growth factors were also seen, suggestive of a role in increased endothelial cell proliferation. These were for serum PDGF in B6C3F1 mice treated with s47 mg/kg/day for 24 months (AA2787), but not in rats treated with s47 mg/kg/day for 18 months (AA2796), and for spleen and bone marrow VEGF and bone marrow bFGF in mice, but not rats (745-03855). However, significant increases in hepatic endothelial cell proliferation (proportion of cells in sections identified as endothelial that showed fluorescence after prior *in vivo* BrdU treatment) sometimes resulted from abnormally low control values and the extent to which a real effect was being measured was not always clear. Additionally, apparently increased labelling of hepatocytes or Kupffer cells was sometimes seen, creating some uncertainty as to the specificity of the response. Another finding of uncertain significance was suppression of respiratory function in mice, with an associated increase in serum bicarbonate.

Overall, there was a strong association between bone marrow megakaryopoiesis, platelet activation, endothelial cell proliferation (within the constraints noted above) and haemangiosarcoma formation in mice, but not rats, and these factors were the subject of detailed assessments by Pfizer (745-03856 and earlier versions 745-03607 and 745-03754). In particular, the possible mode of action was pursued to facilitate inter-species comparisons and help determine the relevance of the mouse findings to humans. It was suggested that alterations of platelet function and an increased megakaryopoiesis resulted in increased exposure of the endothelium to platelet associated growth factors resulting in increased endothelial proliferation and haemangiosarcoma formation. No increased endothelial cell proliferation or haemangiosarcoma formation would then be expected in the absence of changes in platelet function or megakaryocytosis, as in rats, and platelet changes probably serve as markers for the process leading to haemangiosarcoma formation. Although the mechanism(s) involved in altered megakaryopoiesis and platelet function in mice are unknown, one suggestion was an indirect effect of low-grade hypoxia and acid-base imbalances resulting from elicited respiratory depression (745-03856). Further work would, however, be needed to determine whether this was the case.

As noted above, investigations in the pivotal cynomolgus monkey study did not show any effects of pregabalin treatment on ADP-induced aggregation or retrospective blood smear platelet and erythrocyte morphology. Slight *decreases* in bone marrow megakaryocytes were seen with the high-dose of s47 mg/kg/day, which achieved an adequate expected drug ER value (8, sex-combined). Additionally, there was no evidence of vascular proliferative lesions in this study (retrospective evaluation), and together these findings strengthen the view that increased haemangiosarcoma formation is specific to mice, assuming that the proposed involvement of platelet activation is correct. Dermatopathy was a consistent finding in cynomolgus monkeys, and although endothelial cells and thrombosis appeared to be involved in its pathogenesis, the former were characterised by cell damage (above).

In view of the findings in mice, special platelet investigations were carried out in some of the clinical trials, including ADP-induced aggregation, activation (P-selectin expression, annexin V binding) and morphological assessment (Clinical overview, 745-035754). Doses were up to s47 mg/day for 4 weeks or 3 years (morphology only) with no adverse effects being observed.

On balance, the proposed mechanism is considered reasonable. In view of the demonstrated differences between rats, cynomolgus monkeys and (to the extent that examinations have been carried out) humans, and mice, the tumourigenic response in mice is probably not relevant to humans, although this cannot be entirely excluded. It was considered by several external consultants that a single species single tumour elicited by an epigenetic mechanism was unlikely to represent a carcinogenic risk to humans at the therapeutic dose (745-03370, 745-03221).

Reproductive toxicity. Embryofetal development studies showed no effect of treatment in mice with doses achieving high drug ER values (to s47 and a MD NOEL of s47 mg/kg/day (ER value of *ca* s47 in rabbits. The HD used in the rabbit study (ER s47 elicited maternal toxicity and resulted in fetal developmental retardation (reduced weights and ossification) and increased incidences of skeletal and visceral variations. Although these variations were probably incidental, an effect of treatment was inconclusive because this interpretation relied on comparison with historical control values. This also applied to one variation (decreased gallbladder size), for which the litter (but not the fetal) HD incidence was above the limited historical control range.

Reduced maternal weight gain and increased fetal skeletal variations were seen with all doses used in the rat study, which achieved high drug ER values s47 Fetal weights were also decreased (HD), and there were increased incidences of 2 skeletal changes in the MD and/or HD groups (respective ER s47 that were initially classified as malformations (jugal bone fused to maxilla, nasal bones fused). The ER value was s47 t the NOEL for these changes s47 /day). Supplementary studies (AA2621, AA2646, AA2604) confirmed these skeletal effects of treatment and showed that they were normal stages in development (representing closure of the zygomaticomaxillaris and nasofrontalis sutures) whose incidence increased with fetal and pup age to weaning. Treatment-related increases at the normal examination time on gestation day 21 therefore represented advanced ossification and a classification of variation was appropriate. These findings were the subject of an assessment by Pfizer (745-03337), in which it was noted that premature suture closure did not result in facial dysmorphogenesis, a conclusion based *inter alia* on results of the pre- and post-natal study in which dosing was over gestation day s47 . A NOEL was not established for fetal development in the main embryofetal development study, with increased skeletal variations being seen with the LD of s47 s47 /day, although these were relatively minor (ventral tubercle of atlas impaired ossification, extra well formed lumbar ribs, rudimentary cervical ribs). Lower doses of s47 mg/kg/day were used in a further embryofetal development study (AA2621), but skeletal examinations were restricted to the skull. Male-mediated embryofetal toxicity was not observed at doses of up to s47 mg/kg/day (estimated ER values to 17) in another study (AA2603).

Male rat fertility was reversibly impaired by treatment, with a NOEL of s47 mg/kg/day (expected ER value of *ca* 11), associated with decreased sperm counts and quality, but while female rat oestrus cycles were reduced, fertility (pregnancies/mating) was unaffected at doses to s47 mg/kg/day. Post-implantation losses were, however, increased and the overall NOEL was the mid-dose of s47 mg/kg/day, associated with an expected ER value of s47 Increased pre-implantation losses occurred at this dose, although the control value was particularly low and the mid-dose value of 10% was below the mean historical control value of s47 suggesting that this was an incidental finding. Effects on rat sperm quantity and quality were investigated (at a fixed s47 mg/kg/day dose) in some detail in a supplementary study, but it is not clear to what extent reversible impairment was potentially applicable to humans because of the high doses used and (as noted above) the lack of similar findings in cynomolgus monkeys. Semen examinations in the pivotal cynomolgus monkey study did not show any effects of treatment, which may have reflected the fact that the achieved high-dose drug ER value was slightly lower than that at the NOEL for impaired sperm quantity and quality in the rat fertility study s47 or

a species difference. In any case, the lack of effect on sperm in both species at substantially higher than the maximum expected human drug exposure suggests that an effect with the therapeutic dose is unlikely. Analysis of semen from 30 patients in a clinical trial did not show any effects of treatment with 600 mg/day for 3 months (clinical overview). Fertility in rats treated from one week of age is discussed below (*Young animal studies*).

Marked maternal toxicity, including dystocia, failure to deliver and slightly increased gestation length, were seen after treatment of pregnant rats from early gestation through to weaning in a pre- and post-natal study (1960/1962) , but at very high doses (s47 [redacted] mg/kg/day), for which limited data showed drug ER values of >*ca* 50. Stillborn pups were increased and offspring development was also affected, with increased mortality (often with total litter loss), reduced body weights and impaired development and reproductive function being seen. An ancillary study (2123) using the higher of these doses showed that increased stillborn and decreased pup survivals and body weight occurred to a much greater extent with dosing during late gestation (day 17 to parturition), compared with the period of organogenesis or during the first 10 days of the lactation period. A NOEL of s47 [redacted] mg/kg/day was established for developmental toxicity in the main study, while slightly reduced pup weights at weaning were seen with the next s47 [redacted] /day (respective ER values of 2 and 5). In view of the relatively low drug exposure at the NOEL, and the substantial concentrations of pregabalin measurable in milk at these 2 doses s47 [redacted] use in either pregnancy or lactation cannot be recommended.

Young animal studies. Pregabalin is also intended for use in adolescents (12-17 years of age) with epilepsy, although the clinical overview suggested that there were too few patients in the relevant clinical trial to draw meaningful conclusions about the safety profile in this group. Several PO (gavage) studies using neonatal (1 week old) rats were carried out to support pregabalin use in younger subjects, all using a high-dose of 500 mg/kg/day given for at least 7 weeks (AA2518, AA2589, AA2861, AA2772, 2586). This achieved an expected drug ER value s47 [redacted] from a 3 week toxicokinetic study (AA2712) ($AUC_{0\text{-}24\,h}$ values were *ca* 70% higher in 12 week old (AA2715) compared with 1-3 week old rats (AA2712)). No unexpected toxicity was seen in a general toxicity study (AA2518), although pharmacological clinical signs (hyperactivity, bruxism) were observed, and sexual maturation and fertility were unaffected by treatment (2586, AA2772), despite a prolongation of dioestrus (also seen in the adult rat fertility and early embryonic development study).

Hyperactivity and bruxism were also apparent in 2 behavioural neurotoxicity studies (AA2589, AA2861), suggesting that young animals may be more susceptible to pharmacological effects. Other minor group behavioural differences were observed, with testing during the treatment period, together with acoustic startle changes. Males treated with all doses showed reduced mean maximum responses in the first 3 of multiple trials, and in a repeat study in which testing was carried out at 1-2 weeks after the treatment period (to try to distinguish developmental toxicity from pharmacological effects), this was again observed in males at s47 [redacted] mg/kg/day. This particular response was not impaired in females during the treatment period, and mean values tended to be slightly higher, but other variables suggested that female responses may have been affected by treatment. However, in contrast to males, no abnormalities of the acoustic startle response were seen in females after the treatment period, but a decrease in post-weaning maximum response values was seen in female offspring from dams treated with s47 [redacted] mg/kg/day, but not s47 [redacted] mg/kg/day, in a rat pre- and post-natal study. Although the biological significance of these findings is not clear, hearing and ability to physically respond to acoustic stimuli did not appear to be affected, and its relevance to humans is uncertain. The drug ER value at the NOEL of s47 [redacted] day for testing after the treatment period was *ca* 2, and overall, findings in the young animal studies are not considered sufficient to preclude use in adolescents. Studies were,

however, limited in scope because they were restricted to rats, and a more comprehensive investigation could have included young primates.

Impurities. The sponsor seeks approval for the following specification limits for two impurities in Lyrica® *viz* the [redacted] of pregabalin [redacted] in pregabalin drug substance) and the degradant, pregabalin lactam [redacted] in finished product).

The table below shows the calculated PO 'doses' of the impurities achieved in the animal studies *cf* the human 'doses' at the maximal recommended human dose of pregabalin [redacted] mg/kg/day [redacted] day). Also tabulated are the systemic exposures (plasma AUC) to the two impurities measured in the toxicokinetic studies *cf* estimated human exposures.

Study, compound, duration of dosing, and route	Animal dose		Plasma $AUC_{0\text{-}24h}$ (µg.h/mL)	ER if present at proposed specification limit
	Mg/kg/day	*Mg/m²/day*		
SN 1654: R-enantiomer x 13 weeks, PO	[redacted]		[redacted]	[redacted]
SN 1616: Pregabalin lactam x 4 weeks, PO				

* Based on human [redacted] and assuming similar kinetic behaviour for pregabalin and the [redacted] impurities. ER, animal/human exposure ratio; BSA, body surface area

The R-enantiomer showed little or no pharmacological activity *cf* pregabalin, and the only untoward finding in the rat 13 week PO toxicity study (SN 1654) was the occurrence of convulsions in one animal treated with the high-dose of [redacted] kg/day, although as a single case (1/32), the relationship to treatment was not clear-cut. In terms of BSA, the mid-dose of [redacted] mg/kg/day [redacted] mg/m²/day) was equivalent to [redacted] the maximum human intake at the proposed specification limit of [redacted] while the high-dose of [redacted] kg/day was [redacted] the maximum human intake. Plasma [redacted] concentrations were measured in this toxicity study, with similar ER values (AUC) of [redacted] (mid-dose, high-dose, respectively). An additional finding was the lack of clastogenic activity, as assessed by frequencies of micronucleated PCE (SN 1654), and the [redacted] was also inactive in an *in vitro* bacterial gene mutation assay (SN 2472). Overall, these data are considered to have qualified the proposed specification limit [redacted] in pregabalin drug substance).

There were no data on the pharmacological activity of pregabalin [redacted] There were no untoward findings in the rat 4 week PO toxicity study (SN 1616) at doses up to [redacted] In terms of BSA, the high-dose of [redacted] was equivalent to *ca* 30-fold the maximum human intake at the proposed specification [redacted] Plasma lactam concentrations were measured in this toxicity study, with an ER [redacted] As with the [redacted] assays of clastogenic activity *in vivo* (rat micronucleus test; SN 1616) and gene mutation *in vitro* (bacteria; SN 2295) were negative. Overall, these data are considered to have qualified the proposed specification [redacted]

Based on these collective data, there are no nonclinical objections to the proposed specification limits for the two impurities.

PRODUCT INFORMATION

The following changes are suggested:

Pharmacology. Statements under the subheading *Mechanism of action* are supported by the data, but a second paragraph sentence should be slightly altered as: Pregabalin does not interact with either $GABA_A$ or $GABA_B$ receptors; it is not converted metabolically into GABA or a GABA agonist; it is not an inhibitor of acute GABA uptake or degradation.

The first sentence under the subheading *Pharmacokinetics: Distribution* should be slightly altered as: In preclinical studies, pregabalin has been shown to ~~readily~~ cross the blood brain barrier in mice, rats and monkeys. The remaining nonclinical statements under this and the *Metabolism* subheading are supported by the data.

Carcinogenesis, mutagenesis, impairment of fertility.

Carcinogenesis. Two-year dietary carcinogenicity studies with pregabalin were conducted in rats and mice. No increased incidence of tumours ~~were~~ was observed in rats at exposures (plasma AUC) up to 25 times the ~~mean~~ expected human exposure at the maximum clinical dose of 600 mg/day. In mice, no increased incidence of tumours was found at exposures similar to the ~~mean~~ expected maximum human exposure, but an increased incidence of haemangiosarcomas was observed at exposures 6 to 33 times the ~~mean~~ expected maximum human exposure. The precise non-genotoxic mechanism of pregabalin-induced tumour formation is not fully characterised~~; however, pregabalin-induced platelet changes associated with endothelial cell proliferation and tumours in mice are not present in rats or in humans, based on available data.~~ However, available data show that platelet changes associated with formation of this tumour in mice are not seen in rats, monkeys and humans. Although long-term data in humans are limited, ~~there is no evidence to suggest any associated risk in humans.~~ these findings in mice are thought not to pose a risk to humans.

Mutagenesis. Pregabalin is not genotoxic based on results of ~~a battery of~~ *in vitro* and *in vivo* tests. It was not mutagenic in bacteria or in mammalian cells *in vitro*, nor clastogenic in mammalian systems *in vitro* and *in vivo*, and it did not induce unscheduled DNA synthesis in mouse or rat hepatocytes.

NB this subsection should be placed before *Carcinogenesis*.

Fertility.

Preclinical data. In male rats, oral ~~pregabalin~~ administration of high doses of pregabalin resulted in reversible decreased sperm motility and fertility. These were not observed at ~~exposures ≥11~~ exposures (plasma AUC) up to 11 times the ~~mean~~ expected human exposure at the maximum recommended clinical dose of 600 mg/day. There were also no drug-related effects on sperm parameters in a long-term monkeys study ~~given the highest dose for 52 weeks~~ with exposures up to 8 times the expected maximum human exposure ~~recommended clinical dose~~. In female rats, oestrus cycles ~~and diestrus stages~~ were prolonged by high oral doses of pregabalin, but fertility was unaffected, and an increase in post-implantation loss also occurred. No adverse effects were seen at an exposure approximately 50 times the expected maximum human exposure. ~~at ≥27 times the maximum recommended human exposure without effects on fertility~~.

Human data. In a double-blind, placebo-controlled clinical trial to assess the effect of pregabalin on sperm motility, 30 of 46 healthy male subjects were exposed to pregabalin at 600 mg/day for 3

months. Pregabalin did not exhibit detrimental effects on the reproductive function of healthy male subjects, as measured by semen analysis.

Use in pregnancy. (Category B3). LYRICA has not been studied in pregnant women and ~~Studies in animals have shown reproductive toxicity (see Teratogenesis). The potential risk to humans is unknown. Therefore~~ LYRICA should not be used during pregnancy unless the benefit to the mother clearly outweighs the potential risk to the ~~foetus~~ fetus. In a pre- and post-natal study in rats, pregabalin treatment resulted in offspring developmental toxicity at exposures (plasma AUC) ≥5 times the expected human exposure at the maximum recommended clinical dose of 600 mg/day. Offspring development was unaffected at 2 times the expected maximum human exposure.

Labour and delivery. The effects of pregabalin on labour and delivery in pregnant women are unknown. In ~~the~~ a ~~prenatal/postnatal~~ pre- and post-natal development study in rats, pregabalin prolonged gestation and induced dystocia at exposures (plasma AUC) approximately 50 ~~≥47~~ times the ~~mean~~ expected human exposure at the maximum recommended clinical dose of 600 mg/day. These effects were not observed at an exposure that was approximately 12 times the expected human exposure.

Teratogenesis. Pregabalin was not teratogenic in mice, rats or rabbits. ~~Foetal toxicity in rats and rabbits occurred only at exposures >39 times the mean human exposure at the maximum recommended clinical dose of 600 mg/day. In prenatal/postnatal toxicity studies, pregabalin induced offspring developmental toxicity in rats at exposures ≥5 times the maximum recommended human exposure. No developmental effects occurred at 2 times the maximum recommended human exposure.~~ Fetal developmental toxicity was not observed after treatment of pregnant mice and rabbits with oral doses that resulted in respective pregabalin exposures that were 30 times and 17 times the expected human exposure at the maximum recommended clinical dose of 600 mg/day. Increased fetal skeletal variations were seen in rats at oral doses resulting in exposures >17 times the expected maximum human exposure, but lower doses were not tested in a full study.

Use in lactation. It is not known if pregabalin is excreted in the breast milk of humans; however it is present in the milk of rats. Therefore, breastfeeding is not recommended in women taking LYRICA.

CONCLUSIONS AND RECOMMENDATIONS

The most noteworthy nonclinical finding was the occurrence of an increased incidence of haemangiosarcomas in mice, but not rats, in long-term carcinogenicity studies. Pregabalin was not genotoxic in adequate assays, and although the epigenetic carcinogenic mechanism has not been fully elucidated, there was evidence of an association with abnormal platelet function, including increased numbers and activation, and abnormal morphology and aggregation. In view of the role of bone marrow and platelets in endothelial homeostasis and the endothelial origin of haemangiosarcoma, exposure of endothelial cells to elevated levels of endothelial growth factors arising from increased megakaryopoiesis and platelet activation is the most plausible mechanism of the elicited endothelial cell proliferation in mice. There was no evidence of this pattern of changes occurring in long-term studies in rats or monkeys, suggesting that the mechanism may be specific to the mouse.

The available clinical data to date apparently do not show evidence of these platelet changes in humans, and it is considered that this oncogenic finding is unlikely to pose a risk to humans, although such a response cannot be excluded completely. This conclusion would be strengthened

by additional long-term clinical data, and it is noted that the sponsor has undertaken to provide further data concerning assessment of platelet morphology (a marker of platelet activation) to the EMEA as a post-approval commitment.

There were no other findings of concern in the toxicity studies, including no evidence for QT interval prolongation, teratogenicity, or findings in young animal studies that would preclude use in adolescents, although the latter were limited in scope because they were restricted to rats.

Efficacy was demonstrated in models of neuropathic pain and seizures although, despite extensive investigations, the exact mode of action of pregabalin is not clear.

There are no objections on nonclinical grounds to the registration of pregabalin as proposed. The draft Product Information document should be amended as indicated above.

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12. LOCAL TOLERANCE

13. OTHER TOXICITY STUDIES

1. INTRODUCTION

Pfizer Australia Pty Ltd have submitted an application to register pregabalin, a NCE structurally related to L-leucine, γ-aminobutyric acid (GABA) and a registered anticonvulsant, gabapentin (Neurontin). The latter has been used to define the *in vitro* binding characteristics of pregabalin, and as a comparator in several primary pharmacological studies.

1.1 Proposed indications and dosage

Pregabalin is for the treatment of neuropathic pain in adults, and as adjunctive therapy in patients 12 years of age and older with partial seizures with or without secondary generalisation. The proposed starting oral dose for both indications is 150 mg/day, increasing to a maximum of 600 mg/day and given as 2 divided doses. Adolescent patients (12-17 years old) with epilepsy may be dosed as adults.

1.2 Chemical structure and formulation

Pregabalin (code CI-1008) is (S)-3-(aminomethyl)-5-methylhexanoic acid (structure shown in appendix 1). It has a mol wt. of 159.2 and is freely water soluble. Excipients are lactose, maize starch and talc, with the gelatin capsule containing titanium dioxide, sodium lauryl sulphate and colloidal silica.

1.3 International status

Applications for registration of Lyrica® for the treatment of neuropathic pain and epilepsy have been approved in Europe (Centralised Procedure) (July 2004), USA (Approvable Letters) (July & August 2004), Switzerland (Preliminary Approval) (September 2004), and Mexico (September 2004). Applications in a further 11 countries, including Canada and New Zealand, are not yet approved. The FDA has issued a Non-approvable Letter (August 2004) for the indication of generalised anxiety disorder.

2. PRIMARY PHARMACODYNAMICS

In vitro:

Report	Measurements/assays	Results
740-03603	[^{3}H]gabapentin* binding to pig brain membranes, recombinant HEK cell membranes expressing $\alpha_2\delta$-1 (pig) and $\alpha_2\delta$-2 (human)#, brain membranes from R217A transgenic mice&.	K_d (nM): 20 (pig brain), 41 ($\alpha_2\delta$-1), 146 ($\alpha_2\delta$-2), 23 (mouse wild type), 53 (mouse heterozygous), 227 (mouse homozygous). B_{max} (pMol/mg): 0.92 (pig brain), 8.1 ($\alpha_2\delta$-1), 14.5 ($\alpha_2\delta$-2), 0.51-0.56 (mouse).
740-03602	displacement of [^{3}H]gabapentin binding to porcine brain membranes, recombinant HEK cells expressing $\alpha_2\delta$-1 (porcine) and $\alpha_2\delta$-2 (human).	respective K_i values (nM) for pregabalin, R-enantiomer, gabapentin: brain membranes: 19, 239, 28 $\alpha_2\delta$-1: 42, 480, 75 $\alpha_2\delta$-2: 44, 740, 114.
740-03239	inhibition of [^{3}H]gabapentin binding to rat brain membranes.	IC_{50} values (nM): 37 (pregabalin), 620 (R-enantiomer), 80 (gabapentin).

In vitro (continued):

Report	Measurements/assays	Results
740-03614	[^{3}H]pregabalin binding to mouse and rat platelet membranes measured in a displacement assay.	no specific binding observed; K_d = s47 µM for binding to recombinant $\alpha_2\delta$-1 (porcine) cell membranes (positive control).
740-03538	K^+-induced Ca^{2+} influx in rat and cynomolgus monkey neocortical synaptosomes; s47 µM tested.	slight ↓ in both preparations (by *ca* s47 with the R-enantiomer).
770-00311	substance P facilitated/K^+-evoked glutamate release from slices of rat brainstem slices (spinal trigeminal caudal subnucleus).	↓ by *ca* 75% with s47 pregabalin (only concentration tested) but not with R-enantiomer; ↓ 100% with 30 µM gabapentin.
740-03489	K^+-evoked release of [^{3}H]noradrenaline from rat neocortical slices; s47 mM tested.	↓ with maximal inhibition of 40%, IC_{50} for this inhibition range was 11.8 µM; corresponding values for gabapentin were 33% and 8.9 µM; ↓ with 100 µM pregabalin and 100 µM gabapentin were not additive.
740-03578	K^+-evoked/Ca^{2+}-dependent [^{3}H]neurotransmitter (noradrenaline, 5-HT, acetylcholine, dopamine) release from rat discrete CNS area slices; s47 µM tested.	some ↓ release of all neurotransmitters (minimal for striatum dopamine); the largest ↓ was for neocortex noradrenaline and 5-HT (by 36-45%); identical results seen with gabapentin.
740-03537	capsaicin-induced release of immunoreactive substance P and calcitonin gene-related peptide from rat spinal cord slices; samples obtained contralateral and ipsilateral to an inflammatory stimulus (prior treatment with Freund's complete adjuvant).	capsaicin elicited higher release of both mediators from ipsilateral (vs contralateral) slices which was ↓ by s47 µM pregabalin (by 50%); no effect of pregabalin on elicited release by contralateral slices.
740-03516	effects of pregabalin on [^{3}H]GABA uptake and surface/intracellular localisation of the GABA GAT1 transporter protein (Western blot analysis) in neonatal rat hippocampal neurones.	preincubation s47 µM x 2 h) ↑ GABA uptake and redistributed GAT1 transporter from the cytosol to the cell surface; EC_{50} = s47 µM for ↑ GABA uptake (vs 22 µM for gabapentin) and it was apparent after ≥60 min preincubation; additive effect for ↑ GABA uptake with a protein kinase C inhibitor (bisindolylmaleimide II) and brain derived neurotrophic factor but not with Ω-conotoxin or nipecotic acid; pregabalin-induced ↑ GABA uptake abolished by a GAT1 inhibitor (SKF89976A) but botulinum toxin C1 had no effect.
761-00006	effects of pregabalin on MAP kinase signalling pathway in NK1-transfected CHO cells.	↓ substance P-elicited activation of transcription factor Elk-1 s47 additional studies carried out using gabapentin only.

* gabapentin = 1-aminomethyl cyclohexane acetic acid

$\alpha_2\delta$-1, $\alpha_2\delta$-2 = voltage-gated Ca^{2+} channel subunits 1 and 2

& containing an arginine to alanine substitution at position 217 in $\alpha_2\delta$-1

In vivo - analgesia:

Report	Measurements/assays	Results
740-03610	mutant R217A mice* used; tail suspension period of immobility (anxiolytic), period of licking/biting after formalin injection (hyperalgesia) measured; s47 mg/kg PO dose tested.	anxiolysis: ↑ immobility time (wild type) or no effect (homozygous). hyperalgesia: ↓ licking time (wild type, heterozygous) or no effect (homozygous).
770-00297[a]	rats subject to paw formalin or carrageenan injection and effects of SC treatment assessed.	formalin injection: ↓ paw licking/biting time at 11-45 min post-injection (but not at 0-10 min) with pregabalin s47 mg/kg) and gabapentin s47 mg/kg), R-enantiomer showed slight activity at 100 mg/kg; the antihyperalgesic activity of gabapentin shown not to be antagonised by naloxone; 6 daily injections of gabapentin s47 mg/kg) had no effect on final antihyperalgesic activity, unlike morphine s47 s47 mg/kg) for which tolerance developed. carrageenan injection: thermal and mechanical hyperalgesia (paw withdrawal latency) ↓ at 1-2 h post-injection with pregabalin, gabapentin; R-enantiomer had no effect.
740-03479	mouse IP acetic acid writhing test; rat SC hindpaw formalin injection with paw licking/biting time being measured; PO treatment at 2 h prior to testing.	pregabalin and gabapentin at up to s47 /kg were inactive in the writhing test; ED_{50} values for inhibition of formalin response over 11-45 min were s47 g/kg (pregabalin) or s47 g/kg (gabapentin), no effect of treatment was apparent during the early 0-10 min phase.
770-0296[b]	rats subject to hind paw muscle incision resulting in thermal hyperalgesia and tactile allodynia s47 mg/kg SC doses tested.	pregabalin treatment at 1 h prior to surgery ↓ both variables with HD effect lasting at least 72 h; similar but less pronounced effect with gabapentin; HD pregabalin given at 1 h post surgery was effective for only *ca* 3 h.
770-00304	rats subject to hindpaw UV-induced hyperalgesia; s47 mg/kg SC doses tested.	↓ hyperalgesia with pregabalin (all doses) and gabapentin (MD, HD); no activity seen with R-enantiomer.
770-00322	rats subject to sciatic nerve constriction x 14 days or injected with Freund's complete adjuvant (FCA) and used after 24-48 h; electrophysiological measurements carried out; s47 mg/kg IV tested.	chronic nerve constriction: ↓ dorsal root reflex (sural nerve fibres) evoked by a pinch (by 32-61%, significant with MD and HD); maximal ↓ at *ca* 10 min and response normalised by *ca* 60 min. FCA inflammation: ↓ dorsal root reflex evoked by von Frey filaments (by 12-57%, significant for MD and HD).
770-00326	normal rats and rats with diabetic neuropathy (14 days after streptozocin) treated IV with s47 mg/kg; reflex motor responses to pinch stimulus determined over 60 min (electromyographic recordings).	normal rats: ↓ at s47 mg/kg (by *ca* 30%). neuropathic rats: dose-dependent ↓ at s47 mg/kg (by 30%, 40%, 54%).

In vivo - analgesia (continued):

Report	Measurements/assays	Results
770-0295	streptozocin-treated rats assessed for static (von Frey hair pressure) and dynamic (light stroking) allodynia over 0.5-4 h after PO administration.	static allodynia: ↓ with pregabalin s47 mg/kg) and gabapentin s47 ng/kg) but not the R-enantiomer s47 mg/kg). dynamic allodynia: ↓ with pregabalin and gabapentin (30 and 100 mg/kg); R-enantiomer not tested.
770-00312	allodynia (von Frey hair pressure) assessed in sciatic nerve constriction and diabetic neuropathy (streptozocin) rat models; pregabalin was a comparator for the investigation of another compound in this study.	↓ allodynia in both models at 1 h after s47 mg/kg PO or at 0.5-2 h after s47 intrathecal administration of pregabalin.
740-03529 (non-sponsor study)	allodynia (von Frey filament pressure) assessed in a rat neuropathy model (induced by continuous IV vincristine infusion).	↓ allodynia at 30-90 min after IP administration of an s47 mg/kg pregabalin dose.
770-0294	allodynia assessed in rats with sciatic nerve or L5/L6 spinal nerve ligation.	↓ static (von Frey hairs) and dynamic (stroking) allodynia with pregabalin s47 mg/kg PO); only static allodynia ↓ by s47 mg/kg of SC morphine.
740-03589	allodynia (von Frey hairs) assessed in rats treated IM with acid (pH 4.0) saline injections; dynamic allodynia (stroking) was not apparent in this model.	↓ allodynia with pregabalin s47 /kg PO) and morphine s47 /kg SC).
740-03528	rhesus monkeys tested for tail withdrawal latency after immersion in 50 ^{0}C water (a) or 46 ^{0}C water plus local capsaicin injection (b); PO doses of s47 s47 /kg/tested.	treatment a: ↑ latency at 4 h with s47 mg/kg; time course for HD showed peak effect at 3.5-5.5 h. treatment b (allodynia/hyperalgesia): dose-dependent ↑ latency at 4 h with s47 mg/kg.

* containing an arginine to alanine substitution at position 217 in $\alpha_2\delta$-1; similar protein expression in wild type and homozygous mice by Western blotting

[a] Field *et al.* (1997) *Br. J. Pharmacol.*, **121**, 1513-1522

[b] Field *et al.* (1997) *J. Pharmacol. Exp. Ther.*, **282**, 1242-1246

In vivo - epileptic seizures:

Report	Measurements/assays	Results
740-03239	protection from tonic extension seizures induced by maximal electroshock; summarised results from 4 studies.	ED_{50} values (mg/kg). mouse (120 min post-treatment): 20 (IV/PO), 0.65 (IV, low intensity shock), >300 (IV R-enantiomer, 0/8 protected). rat (240 min post-treatment): 1.5-2.2 (IV/PO), >100 (IV R-enantiomer, 2/8 protected).
740-03225	rats treated IV with s47 g/kg of [^{14}C]pregabalin; plasma, neocortex, cerebellar radioactivity concentrations and protection against maximal electroshock measured at intervals over 0.5-14 h.	maximal protection at 2 h (9/10), highest brain concentration over 1-2 h (*ca* 1 µg/g); s47 mg/kg of gabapentin gave a peak brain concentration of *ca* 4 µg/g at 1 h but lower protection (4/10).

In vivo - epileptic seizures (continued):

Report	Measurements/assays	Results
740-03214	protection against pentetrazol-elicited clonic threshold seizures in mice.	respectively 20%, 50%, 80% of mice protected with s47 [redacted] mg/kg PO.
740-03365	protection against audiogenic seizures in DBA/2 mice (genetically susceptible); 2 h pretreatment.	ED_{50} values (mg/kg PO): 2.7 (pregabalin), >300 (R-enantiomer), 12.5 (gabapentin).
740-03551	effect of flumazenil (benzodiazepine antagonist) on protection against audiogenic seizures in DBA/2 mice.	protection by s47 [redacted]g/kg PO of pregabalin and 20 mg/kg PO of gabapentin unaffected by s47 [redacted]ng/kg IP of flumazenil; protection was also afforded by s47 [redacted]ng/kg of PO diazepam, which was abolished by flumazenil; similar results were obtained in a test for anxiolytic-like activity in rats.
740-03216	protection from maximal electroshock tonic extension seizures in mice after single or 5 daily PO treatments with s47 [redacted] mg/kg, or 10 daily doses with s47 [redacted] mg/kg.	no ↑ protection seen with multiple dosing.
740-03614	mutant R217A mice used; protection against maximal electroshock measured; IP doses of s47 [redacted] /kg tested.	approx. ED_{50} values (mg/kg): 7.8 (wild type), 24.7 (heterozygous), 19.7 homozygous).
740-03222	protection against seizures induced by multiple electrical stimulation (1 s or 10 s) of the rat hippocampus.	ED_{50} values (mg/kg IP) for complete protection: rearing seizures: 9.0 (10 s), 9.6 (1 s). behavioural seizures: 57 (10 s), 26 (1 s). afterdischarge suppression: >200 (10 s), 62 (1 s).
740-03518	electroshock seizure duration and paired-pulse inhibition in dentate gyrus of anaesthetised rats investigated; treatment was by IP injection.	pregabalin ↓ pulse-paired inhibition (30 and 100 mg/kg) and ↓ lengthening of seizure discharge duration (maximal dentate activation, MDA) with repeated stimulation s47 [redacted] mg/kg); similar results obtained with gabapentin; nimodipine s47 [redacted] /kg) ↓ MDA but slightly ↑ pulse-paired inhibition at 10 mg/kg.
740-03108	protection against maximal electroshock seizures assessed in rats at 4 h after single or multiple (0, 8, 16, 24 h) IV administration of s47 [redacted] mg of pregabalin.	no difference between single and multiple dosing observed.
740-03136	effects in a rat strain (GAERS) exhibiting spontaneous seizures (genetic absence epilepsy); spike and wave discharges measured; treatment by IP injection.	no effects observed with s47 [redacted] ng/kg, ↑ spike and wave discharges s47 [redacted] /kg, ↑ also seen with s47 [redacted] kg of gabapentin.

In a composite study (**740-03576**), pregabalin and a series of structural analogues were tested for binding to pig brain membranes *in vitro* (competitive assay using [^{3}H]gabapentin) and *in vivo* activity in rat carrageenan thermal hyperalgesia, DBA/2 mice audiogenic seizure and rat Vogel conflict (anxiolytic) tests. PO treatment (30 mg/kg) was at 60 or 120 min prior to testing and there were significant correlations between *in vitro* binding (log IC_{50} values) and activity in all 3 *in vivo* tests. Respective pregabalin, racemate and R-enantiomer binding IC_{50} values were s47 [redacted]

3. SECONDARY PHARMACODYNAMICS

In vitro:

Report	Measurements	Results
740-03076	pregabalin and R-enantiomer s47 screened for receptor binding activity (38 radiolabelled ligand displacement assays).	no activity observed, including binding to $GABA_A$ and $GABA_B$ (rat), benzodiazepine (bovine), opioid (rat, guinea pig) receptors.
740-03547	binding to recombinant human $GABA_B$ receptors using a ^{3}H-labelled GABA antagonist (CGP54626A); 2 cell lines used expressed the R1a/R2 or R1b/R2 receptor subtypes; rat cortical membranes also used.	no pregabalin or gabapentin binding; K_i values (μM) for GABA were 30.8 (R1a/R2), 29.5 (R1b/R2), 1.3 (brain membranes).
740-03548	binding to recombinant human cannabinoid CB1 receptor and rat cortical membranes using a ^{3}H-labelled agonist (CP 55,940).	no pregabalin or gabapentin binding; K_i for CP 55,940 was *ca* s47
770-00350	binding to human recombinant cannabinoid CB1 and CB2 receptors using [^{3}H]WIN 55212-2 ligand.	no pregabalin binding
740-03545	affinity for neurotransmitter reuptake sites in rat brain synaptosomal preparations using ^{3}H-labelled dopamine, noradrenaline, 5-HT.	no pregabalin and gabapentin affinity.
761-00012	effect on rat brain GABA transaminase activity.	no significant inhibition with s47 pregabalin or R-enantiomer; slight ↓ with s47 gabapentin (by 32%).
740-03515	*ex vivo* neonatal rat optic nerve GABA content at 150 min after IP treatment s47 mg/kg).	no effect with pregabalin, gabapentin; ↑ GABA (>10x) with vigabatrin (GABA transaminase inhibitor).
760-00132	effect on human platelet, U-937 cell and LPS-stimulated J774A cell cyclooxygenase activity; indomethacin positive control included.	no inhibition s47
740-03220	voltage-clamped Na^+ currents in cells expressing recombinant rat brain type IIA Na^+ channels (α subunit).	no effect of s47 μM pregabalin; ↓ with 100 μM lidocaine.
740-03519	voltage-gated ion channel currents in neonatal rat superior ganglion neurones, CHO cells expressing rat brain type IIA Na^+ channels (α_1 subunit), HEK cells expressing Ca^{2+} channels (human α_{1B}, rabbit muscle $\alpha_2\delta$, human neuronal β_2 subunit).	no ↓ with s47 μM pregabalin
740-03539	GABA-evoked currents in voltage-clamped rat fetal cortical neurones.	no effect of pregabalin (100 μM); ↑ with diazepam (EC_{50} = s47 and hexobarbitone (EC_{50} = s47 μM).
740-03517	intracellular recordings in rat hippocampal slice CA1 region pyrimidal cells.	s47 μM pregabalin had no effect on NMDA* or AMPA mediated synaptic transmission (epsp), GABA mediated inhibitory synaptic transmission (ipsp), long-term potentiation in stratum radiatum cells over 30 min.

* NMDA = N-methyl D-aspartate, AMPA = 3-amino-2-(4-chlorophenyl)propylsulfonic acid

In vivo:

Report	Study type and experimental details	Results
740-03464	anxiolytic-like activity measured in mice (tail-suspension) and rats (elevated X-maze, Vogel conflict test); PO treatment at 2 h prior to testing.	activity demonstrated in the mouse tail-suspension test and rat Vogel conflict test but not the rat elevated X-maze test.
770-01316	anxiolytic-like activity (ALA) assessed in 2 rat models; SC treatment at 40 min prior to testing.	elevated X-maze behaviour: ALA with s47 mg/kg of pregabalin. conflict test with punishment: ALA with s47 mg/kg of pregabalin; slight ALA with s47 mg/kg of R-enantiomer.
740-03526	anxiolytic-like activity assessed in a rhesus monkey punished response model.	↑ punished responses with PO pregabalin and s47 s47 kg) consistent with anxiolytic activity; similar results with PO alprazolam s47
740-03483	Sidman avoidance behaviour (measure of anti-psychotic activity) assessed in 6 ♂ squirrel monkeys (cross-over design); PO treatment with s47 /kg s47 kg of haloperidol; hourly measurements for 6 h after dosing.	↑ response rate with pregabalin (MD, HD); ↓ response rate and inhibition of avoidance responding with haloperidol; concluded overall that pregabalin lacks antipsychotic activity seen with haloperidol.
760-00177	anti-inflammatory activity assessed in reactivation arthritis elicited in Lewis rats by systemic treatment with streptococcal cell wall components after prior intra-articular administration; PO treatment with s47 s47 kg/day x 3 days (first dose at 1 h prior to systemic challenge).	↓ paw oedema, no effect on synovial fluid neutrophil infiltration, ↓ ankle joint substance P and calcitonin gene related peptide, ↓ paw thermal hyperalgesia; similar effects seen with gabapentin.
760-00178	type II collagen-induced arthritis in DBA/1 mice measured; PO prophylactic treatment bid s47 mg/kg) starting at 3 days prior to collagen immunisation.	delayed time to onset of arthritis with HD, no difference in severity.
760-00138	effect on indomethacin-induced gastric lesions investigated; PO/IP s47 mg/kg) or intracisternal s47 mg/kg) treatment at 1 h prior to indomethacin.	↓ lesion area with all administration routes, no effect on gastric mucosal PGE_2 content, similar results with PO gabapentin (other routes not investigated).
6051-00002	rats received a trinitrobenzene sulfite (TNBS) injection into the proximal colon; pain response on day 7 assessed by measurement of abdominal contractions with progressive balloon inflation.	↑ TNBS-induced allodynia threshold with SC pregabalin s47 /kg) and morphine (0.03-1 mg/kg) at 0.5 h before testing; naloxone abolished morphine but not pregabalin activity; pregabalin ED_{50} values were s47 mg/kg (PO 1 h prior to testing) s47 mg/kg (SC, 0.5 h prior to testing); pain threshold in normal rats unaffected by SC pregabalin s47 /kg) but ↑ by morphine (0.1-1 mg/kg).
6051-00003	rats received IP injections of bacterial endotoxin and after 12 h abdominal contractions elicited by rectal distension were measured by electromyography.	↓ abdominal contractions with s47 mg/kg of PO pregabalin given at 2 h before rectal distension.

In vivo (continued):

Report	Study type and experimental details	Results
6051-00004	clinical pain responses assessed in rats after a colonic formalin injection; treatment with pregabalin at 30 min (SC) or 5 min (intracerebroventricular, ICV; intrathecal, IT) prior to formalin.	↓ clinical response with [s47] /kg SC (by 69%), [s47] rat ICV (by 44-99%), 100-300 µg/rat IT (by 42-60%).
6051-00008	rats received colonic infusion of glycerol and abdominal contractions were measured by electromyography; PO pregabalin treatment at 1.5 h before glycerol infusion.	↓ abdominal contractions with [s47] kg of PO pregabalin (by 27-70%).
6051-00009	rats subject to a 2 h restraint stress session and 20 min later abdominal contractions elicited by a graded rectal distension were measured; PO pregabalin treatment just prior to stress session.	↓ abdominal contractions with [s47] kg (by 64-76%).
6051-00005	abdominal contractions elicited by graded rectal distension measured by electromyography in guinea pigs; PO pregabalin treatment at 2 h before testing.	↓ abdominal contractions at [s47] /kg (by 68%); no effect at [s47] /kg and tendency for ↓ at [s47] mg/kg.

4. SAFETY PHARMACOLOGY

Report	Study type and experimental details	Results
745-02928*	neurofunctional evaluation in ♂ mice and rats; IV treatment with [s47] /kg; observation of clinical signs/behaviour over 60 min, at which time specific tests/measurements carried out (e.g. reflexes, co-ordination, grip strength, body temperature, locomotion).	mice: urine staining/hypoactivity (HD), ataxia (HD), ↓ body temperature (LD, HD), ↓ vertical movements/stereotypy time (LD, HD); 5 min plasma concentrations were 96 (LD) or 499 (HD) µg/mL. rats: hypoactivity (LD, HD), ataxia (HD), ↓ body temperature (LD, HD), ↓ vertical movements/stereotypy time/distance moved (LD, HD), ↑ tail analgesia (HD), ↑ foot splay distance (LD, HD); 5 min plasma concentrations were 104 (LD), 591 (HD) µg/mL.
740-03527	sleeping monitored in ♂ rats with indwelling EEG, EMG and brain thermistor electrodes; PO treatment with [s47] mg/kg just prior to light cycle onset; recordings over 23 h.	↑ non-rapid eye movement sleep and EEG slow wave activity at [s47] mg/kg, ↓ rapid eye movement sleep at [s47] mg/kg, no change in sleep cycle length or latency to sleep; results considered consistent with hypnotic-like activity.
740-03215	ataxia, muscle tone, righting reflex assessed in ♂ rats at 2.5 h after PO treatment with [s47] [s47]	↓ righting reflexes in 1/8 LD, 3/8 MD, 8/8 HD rats.
770-01317	♂ rat sensory motor co-ordination assessed in a beam walking test at intervals after PO treatment with [s47] mg/kg.	marked ↑ crossing time and number of foot slips (all doses), ↑ rat falls (MD, HD); slight activity with 300 mg/kg gabapentin.
740-03217	♂ mice tested for ataxia at 0.5-3 h after IV [s47] or PO [s47] treatment.	no consistent effect of treatment.

Continued:

Report	Study type and experimental details	Results
740-03074	♂ mice tested for motor activity, and clinical signs observed, after IV [redacted] or PO [redacted] treatment.	↓ activity with IV and PO HD.
740-03472	♂ rats and mice tested for locomotor activity and ability to hang on to an inverted screen at 2 h after PO administration.	rats: ↓ distance travelled at 100-300 mg/kg, not seen with gabapentin, also apparent with lorazepam [redacted] ↑ falls in inverted screen test [redacted], not seen with gabapentin, also apparent with lorazepam [redacted] mg/kg). mice: no significant effects on distance travelled with pregabalin or gabapentin [redacted] mg/kg), ↓ with lorazepam (≥1 mg/kg); no falls in inverted screen test with pregabalin or gabapentin [redacted] mg/kg), ↑ with lorazepam (≥3 mg/kg).
7740-03115	cardiovascular responses in ♂ rats (2 groups of 10); PO treatment sequentially with [redacted] ng/kg on 3 consecutive days, or water vehicle; heart rate and mean arterial blood pressure measured over 20 h post-dosing (48 h after last dose).	no effect of drug treatment observed.
745-02986*	cardiovascular and renal responses in 8 ♂ Wistar rats (cross-over design); IV treatment with [redacted] mg/kg; heart rate, arterial blood pressure measured hourly over 1-24 h; 0-24 h water consumption, urine volume and electrolyte excretion measured.	cardiovascular: no change with LD; slightly ↑ heart rate with HD over 2-9 h (by 9-19%), but not subsequently; qualitative ECG waveforms unaffected by treatment. renal: no change with LD; ↑ water consumption (by 29%) and urine volume (by 82%) with HD.
742-00010	cardiovascular responses in 4 ♂ dogs; each dog received PO treatment with an empty gelatin capsule or [redacted] of solid drug; measurements at 1 h prior to and for 6 h after treatment.	no drug-related changes in mean arterial blood pressure, heart rate, cardiac output, total peripheral resistance, left ventricular dP/dt; mean plasma pregabalin concentrations were [redacted] /mL at 1-6 h.
760-00073	pulmonary function in anaesthetised mongrel dogs (2/group); [redacted] mg/kg infused IV over 50 min.	no data presented, but no effects of treatment said to have been observed.
745-02988*	cardiovascular responses in 3 ♂ cynomolgus monkeys (cross-over design); IV treatment with [redacted] /kg; hourly recordings over 1-24 h.	no effects of drug treatment on heart rates or qualitative ECG waveform; slight tendency for ↓ mean arterial blood pressure (by 6-11% for means over all time points).
6051-00006	♂ rat gastric emptying and intestinal transit measured using milk meal spiked with [^{51}Cr]EDTA; PO treatment with [redacted] at 2 h before the meal (10/group); rats killed at 15 min after the meal for measurement of stomach and small intestinal (10 segments) radioactivity.	dose-related ↓ in gastric emptying (by 12-64%) and intestinal transit (by 10-37%); MD activity was unaffected by [redacted] kg of SC naloxone.
6051-00007	♂ rat colonic transit of ^{51}Cr sodium introduced via an indwelling proximal colon catheter; 2 groups of 6 rats treated PO with saline and [redacted] kg or saline and 100 mg/kg, separated by one week; faeces collected at hourly intervals until radioactivity not measurable.	↑ mean retention times with LD (by 51%) and HD (by 172%).

* GLP-compliant study

5. PHARMACODYNAMIC DRUG INTERACTIONS

No studies.

6. PHARMACOKINETICS

6.1 Method of analysis

Plasma pregabalin was generally measured by an s47 method, with LLQ of s47 µg/mL and validated for mouse, rat, rabbit and cynomolgus monkey samples. ^{14}C-pregabalin was labelled as shown in appendix 1.

6.2 Absorption and plasma kinetics

Study	Species/ group size	Sampling times	Dose (mg/kg) and route*	$AUC_{0-\infty}$ (µg.h/mL)	C_{max} (µg/mL)	F(%)	other values
764-03880	B6C3F1 mice, 3/sex/ sample time.	predose, 9 intervals over 0.25-24 h (plus 2 min after IV).	50 PO (a) 50 IV	s47		94 -	t_{max} = 0.25 h $t_{1/2}$ = 3.4 h; Cl = 627 mL/h/kg; Vdss = 1.04 L/kg
764-02203	♂ Wistar rats, 5/route.	predose, 10-12 intervals over 5 min (IV) or 0.25 h (PO) - 48 h.	50 PO (b) 50 IV			83 -	t_{max} = 0.6 h $t_{1/2}$ = 3.9 h; Cl = 174 mL/h/kg; Vd = 0.99 L/kg
764-02204	♂ Wistar rats, 5-6/dose.	predose, 9 intervals over 0.5-48 h.	PO (c): 5 25 50 100 150				t_{max} = 0.5-1.2 h
764-02299	cyno. monkeys, 3/sex[&].	predose, 12-13 intervals over 0.25 h (IV) or 0.5 h (PO) to 48 h.	PO (d): 10 25 50 100 25 IV			93 70 76 41 -	t_{max} = 0.9-2.3 h $t_{1/2}$ = 5.8 h; Cl = 106 mL/h/kg; Vd = 0.84 L/kg
361 (memo)	rhesus monkeys, 4 ♂[&].	predose, 1, 2, 4, 6, 8 h.	a) 100 PO (e) b) 100 PO (e) on day 1 300 PO (e) on day 7				t_{max} =4.5 h t_{max} =6.5 h t_{max} = 6.0 h

* PO vehicle = 0.5% methylcellulose (a), saline (b), 5% dextrose (c), water (d), not stated (e)
[#] 0-24 h [$] 0-8 h
[&] cross-over design

6.3 Distribution

In vitro:

Report	Study details	Results
764-02316	binding of pregabalin [s47] µg/mL, 2 h at 37 °C) to rat, cynomolgus monkey and human plasma proteins determined by ultracentrifugation.	no binding measurable.
764-03885	[^{14}C]pregabalin [s47]) plasma/RBC distribution in mouse, rat, dog, cynomolgus monkey and human blood measured after 3 h incubation at 37 °C.	no dose-dependency observed; approx. partition coefficients (Kp) were 0.81 (mouse), 0.78 (rat), 0.71 (dog), 0.79 (cynomolgus monkey), 0.69 (human).

In vivo:

Report	Study details	Results
764-03718	♂ B6C3F1 mice treated PO with [s47] kg of [^{14}C]pregabalin and single animals were killed at 1, 2, 4, 8, 24, 48 h for quantitative whole body autoradiography.	radioactivity was widely distributed, with peak concentrations (µg eq/g) at 1 h; except for the pancreas (30.1) and kidneys (excretory organ) values were ≤ for blood (4); the lowest peak values were for fat (<LLQ of 0.15), brain (0.9) and seminal vesicles (0.5); radioactivity was <LLQ at 8 h, except for the pancreas (0.23).
764-02227	Wistar rats treated PO with [s47] g/kg of [^{14}C]pregabalin and 1/sex killed at 1, 2, 4, 6, 24, 48, 96, 192 h for quantitative whole body autoradiography.	radioactivity was widely distributed, with the highest concentrations at 1-2 h (exception was the lens, 4-8 h); peak values (♂/♀, µg eq/g) were ≤ for blood (11.4/11.2) except for the kidneys (excretory organ) and pancreas (51.6/45.2); the lowest peak values were measured in the brain (2.7/3.0), lens (1.7/1.2) and fat (3.1/2.6); concentrations were mainly <LLQ (0.03) at 48 h.
764-02359	pregnant Wistar rats (GD 19) treated PO with [s47] animals killed at 1, 2, 4, 8, 24 h for quantitative whole body autoradiography.	the peak fetal concentration (µg eq/g) was higher than for maternal blood (18.7 at 2 h vs 10.5 at 1 h); high peak concentrations were measured in fetal brain (14.3) and fetal liver (18.5); the 24 h fetal concentration was still higher than for maternal blood (0.19 vs 0.04); the peak maternal pancreas concentration was very high (69.2 at 1 h).
764-02352	cynomolgus monkeys treated PO with [s47] [s47] [^{14}C]pregabalin and 1/sex killed at 4 or 10 h for quantitative whole body autoradiography.	radioactivity was widely distributed, with higher concentrations (♂/♀, µg eq/g) at 4 h; except for testes and epididymides (16.9, 21) and kidneys (excretory organ) 4 h concentrations were ≤ blood values (12.6/18.2); low concentrations measured in the brain (3.5/4.1), lens (1.6/0.9), pituitary (6.6/10.1), skin (6.4/8.3) and thyroids (4/3.9).

6.4 Metabolism

In vitro:

Report	Study details	Results
764-02235	[^{14}C]pregabalin incubated with cofactor-supplemented rat, dog cynomolgus monkey and human liver cytosol and microsomal preparations [s47] uM plus [s47] mg protein/mL x 120 min at 37 °C).	no metabolism observed.
764-03070	[^{14}C]pregabalin incubated with rat, dog, cynomolgus monkey and human hepatocytes [s47] x 180 min at 37 °C).	trace N-methyl derivative detected in rat, dog, human samples.

In vivo:

Study/report	Study details	Results
PDM-00157	5 B6C3F1 mice treated PO with [s47] mg/kg of [^{14}C]pregabalin and pooled urine examined for metabolites.	urinary radioactivity recoveries were 87% (0-24 h), 93% (0-48 h) of the dose; 92% of 0-24 h radioactivity associated with unchanged drug, and 2% with (possibly) the N-methyl derivative.
PDM-00272	♂ CD-1 and ♂ B6C3F1 mice treated with 1000 mg/kg/day of pregabalin x 2 weeks followed by [s47] kg of [^{14}C]pregabalin; pooled 0-24 h urine (10/strain) and 4 h and 10 h plasma (20/sample time/strain) examined for metabolites.	*urine*: 0-24 h recoveries of radioactivity were 98% (B6C3F1) or 87% (CD-1) of the dose; unchanged drug associated with 93% of radioactivity in both strains, and 2% with the N-methyl derivative; some other trace metabolites also present. *plasma*: all radioactivity associated with unchanged drug.
764-03127	Wistar rats (6/sex) treated PO with [s47] kg of [^{14}C]pregabalin and plasma (1 and 6 h) and 0-24 h urine examined for metabolites.	*plasma*: virtually all radioactivity associated with unchanged drug. *Urine*: unchanged drug (90% of radioactivity), N-methyl derivative (2.5%) and 3 minor unidentified components present.
764-02225	Wistar rats (2/sex), beagle dogs (1/sex) and cynomolgus monkeys (2 ♂) treated PO with [s47] ng/kg of [^{14}C]pregabalin and 0-24 h urine examined for metabolites.	*rat*: unchanged drug and 3 minor metabolites (4.8%, 2.5%, 1.7% of radioactivity). *dog*: unchanged drug, one major metabolite (44.6% of radioactivity) and 3 minor metabolites (3.0%, 1.5%, 0.9% of radioactivity); major metabolite identified as the N-methyl derivative (**764-02260**). *cynomolgus monkey*: >99% of radioactivity was unchanged drug.
764-03395	cynomolgus monkeys treated PO with [s47] mg/kg of [^{14}C]pregabalin; 0-24 h urine and 2 and 8 h plasma examined for metabolites.	*urine*: unchanged drug (92.9% of radioactivity) and a trace unknown metabolite (0.4%) present. *plasma*: only unchanged drug present.

In vivo (continued):

Study/report	Study details	Results
764-02317	selected plasma samples from mouse (**1556**), rat (**1554**), rabbit (**1907**), cynomolgus monkey (**1544**) toxicity studies examined for the presence of the R-enantiomer (HPLC after derivatisation, LLQ = s47 µg/mL).	no R-enantiomer measured; plasma pregabalin concentrations were s47 µg/mL (mouse), s47 µg/mL (rat), s47 µg/mL (one rabbit), s47 µg/mL (cyno. monkey).
1578*	B6C3F1 mice treated with s47 s47 mg/kg/day by dietary administration x 13 weeks; liver microsomal protein, cyt. P450, NDMA, EROD, PROD# measured.	slightly ↓ microsomal protein (by 13%) and ↑ cyt P450 (by *ca* 50%) with HD; enzyme specific activities (nmol/min/mg protein) unaffected by treatment.
1554*	Wistar rats treated with s47 s47 mg/kg/day by dietary administration x 4 weeks; liver microsomal preparation protein, cyt. P450, AH, NDMA, EROD, PROD# measured at termination and after a 4 week recovery period (5/sex/group); Western blot analysis of selected samples for CYP 1A1, 2B1/2, 2E1 carried out.	reversible ↑ generally s47 mg/kg/day but not always significant for cyt P450 (by 30-107%), AH (by 64-427%), NDMA (53-164%), EROD (by 81-253%), PROD (by 93% to 31x control value); Western blot: ↑ 2B1/2, slight ↑ 2E1, 1A1 not detectable.
1537*	Wistar rats treated with s47 s47 mg/kg/day x 2 weeks, by gavage or dietary administration; liver microsomal protein, cyt. P450, AH, ED, NDMA, EROD, PROD# measured (5/sex/group).	similar results were obtained after gavage and dietary administration; the largest ↑ was for PROD in ♂ (up to 18x increase with the HD), with non-significant ↑ of 5-7x in ♀.
1929*	cynomolgus monkeys treated PO with s47 mg/kg/day; liver microsomal preparations protein, cyt P450, EROD, ED# measured (4/sex/group).	no effects of treatment.

* toxicity studies (see section 8)

NDMA = nitrosodimethylamine N-demethylase, EROD = ethoxyresorufin O-deethylase, PROD = pentoxyresorufin O-dealkylase, AH = aniline hydroxylase, ED = erythromycin demethylase

6.5 Excretion

Radioactivity:

Report	Species and group size	Dose (mg/kg)& and route	Collection time	Urine/cage wash*	Faeces*	Total*
764-03127	Wistar rats, 6/sex	25 PO	0-96 h	95	4	99
764-03395	cynomolgus monkeys, 3/sex	10 PO	0-96 h	83.6/7.4	4.9	95.9

& ^{14}C-labelled * % of the dose

6.6 Pharmacokinetic drug interactions

Pregabalin (40, 200, 1000 μM) had no effect on reactions catalysed by common human microsomal CYP isoforms *in vitro* (**764-03016**). These were R-warfarin 6-hydroxylation (1A2) coumarin 7-hydroxylation (2A6), S-warfarin 7-hydroxylation (2C9), S-methoin 4'-hydroxylation (2C19), d-methorphan O-demethylation (2D6), p-nitrophenol to p-nitrocatechol conversion (2E1) and R-warfarin 10-hydroxylation (3A4).

6.7 Other pharmacokinetic studies

Report	Measurements	Results
764-03670	Absorption of [^{14}C]pregabalin in a 2 h single-pass rat *in situ* intestinal perfusion model; s47 μM solution perfused at 0.1 mL/min x 120 min.	absorption of 32x10^{-6} cm/s measured vs respective values 84.6, 30.2, 19.1 x10^{-6} cm/s for propranolol, metoprolol, atenolol.
761-00007	characteristics of cellular transport of pregabalin into CHO and fetal/neonatal rat neuronal cells investigated; ^{3}H-labelled substrates used.	pregabalin competitively ↓ leucine uptake by CHO cells with K_i value of 86 μM (281 μM for R-enantiomer); uptake of pregabalin and gabapentin into cortical astrocytes, cortical neurones, cerebellar granule cells was similar and > than for leucine; efflux of pregabalin from CHO cells was <than for leucine; pregabalin preincubation (100 μM x 1 h) or co-treatment (10 mM) had no effect on initial GABA uptake by cortical neurones.

6.8 Pharmacokinetics in humans

Clinical pharmacology summary data*:

Study	Treatment	C_{max} (μg/mL)	$AUC_{0\text{-}24\,h}$ (μg.h/mL)	Other values
1008-002	s47 PO q8h s47 g PO q12h (8-11 normal subjects treated for 2 weeks).	s47	s47	$t_{1/2}$ = 6.3 h, C_{min} = 3.4 μg/mL $t_{1/2}$ = 6.7 h, C_{min} = 2.7 μg/mL steady states achieved in 24-48 h
1008-005	single 100 mg [^{14}C]pregabalin PO dose (6 normal subjects).			radioactivity recoveries (% of the dose): faeces (<0.1), urinary unchanged drug (89.9), urinary N-methyl derivative (0.9), urinary unknown (0.4).

* there were no data for IV administration

7. SINGLE DOSE TOXICITY

7.1 Rodents

GLP studies were carried out by Parke-Davis, Mississauga, Ontario, Canada over Aug.-Dec. 1992. Except for the young rat study (**2509**), animals were either observed for 14 days, after which they were necropsied, or were killed after 24 h (IV) or 48 h (PO) for limited plasma chemistry measurements and histological examinations.

Study	Species/strain	Dose (mg/kg)*, route and group size	Maximum non-lethal dose (mg/kg)	Findings Document 1
1522	B6C3F1 mice	s47 PO (2 ♀) s47 PO (3/sex)[&]	s47	♂ transient mild hypoactivity.
1514	Wistar rats	s47 PO (2 ♀) s47 PO (3/sex)[&]		transient mild hypoactivity, diarrhoea, ♀ urine staining.
1526	B6C3F1 mice	s47 (2 ♀) s47 (3/sex)[&]		no effects of treatment.
1527	Wistar rats	s47 (2 ♀) s47 (3/sex)[&]		transient hypoactivity, urine staining, mild ataxia.
2509[#]	7 day old and 21 day old Wistar rats.	s47 PO (10/sex)		no clinical signs, ↓ BWG over first week with all doses, thymic red discolouration at necropsy (5/20 HD, 7 day old rats only).

* PO vehicle = 0.5% methylcellulose
[&] killed after 24 h or 48 h
[#] non-GLP study (July-Aug. 1999)

7.2 Cynomolgus monkeys - dose escalation

Study	Group size	Daily dose (mg/kg)* and route	Findings
1846[&]	1/sex (total)	s47 PO on days 1-4, untreated on days 5-7, s47 s47 PO on days 8-11.	no deaths; diarrhoea/soft faeces at ≥800 mg/kg; BW and terminal ophthalmology, body temperature, blood pressure, ECG, haematology and myelograms, serum chemistry, urinalysis unaffected by treatment (quantitative measurements compared with pretest values), and no obvious changes in organ weights and histology (testes/epididymides hypospermia and prostate/seminal vesicle hypoplasia consistent with immaturity).

* 0.5% methylcellulose vehicle [&] GLP-compliant (May 1993)

8. REPEATED-DOSE TOXICITY

8.1 Mice

Pivotal study*:

Study No. and date: **1578**, July-Oct. 1995 Laboratory: Parke-Davis, Mississauga, Canada GLP compliance: Yes (but not signed by study director)				
Dosing route, frequency and duration: dietary x 13 weeks Strain and group size: B6C3F1, 10/sex[&]		Findings ♂/♀[#]		
Dose (mg/kg/day)**:	0	s47 [redacted]		
Mortality (drug-related)[m]	NA	-/2	1/1	-/2
Clinical signs: cage urinary crystalline residue[s]	-	-	-	+/+
BWG[f]	NA	↑48/59	↑27/47	↑10/13
Ophthalmology	NA	-	-	-
Haematology: platelet volume	NA	↑16/11	↑16/24	-/↑20
Myelograms	NA	NE/-	NE/-	NE/-
Serum chemistry	NA	-	-	-
Urinalysis: NE[u]				
Organ weights (body weight-relative): kidney	NA	↓4/-	↓8/-	↓7/↑11
thymus	NA	-/↓30	-/↓38	-/↓38
Gross pathology: renal pelvic focus	-	-	-/1	-/3
Histopathology[h]: renal cortical tubular basophilia/dilation	-/2	NE/-	NE/1	1/5
adrenal X-zone vacuolation[a]	-	NE/6	NE/10	-/9

* range-finding study for carcinogenicity studies
** actual intakes were similar to nominal values
[#] quantitative values are *terminal* % changes from vehicle controls or incidences
- no changes or findings, NA not applicable, NE not examined
[&] plus an additional 18/sex for toxicokinetic measurements
[m] deaths all assigned to toxicokinetic subgroups and may have been incidental, cause of death not known
[s] shown to be pregabalin by s47 [redacted]
[f] associated with sporadic ↑ food consumption
[u] urine only examined for crystals, before and after centrifugation, with negative results
[h] routine examination of control and HD mice
[a] mild-moderate, all ♀ controls showed minimal grade

Other studies:

Study details	Strain and group size	Route	Duration (wk)	Dose (mg/kg/day)	Findings
SN: **1556** Date: Mar. 1994. Laboratory: Parke-Davis, Mississauga, Ontario, Canada. GLP: Yes.	B6C3F1, 10/sex*	diet	2	s47 [redacted]	*Similar to the pivotal study*: ↑ ♂ platelet volume, renal cortical tubular basophilia (4/20 HD, minimal). *Additional findings*: ↓ BWG (MD, HD), sporadic ♂ urine staining (MD, HD), ↑ erythroid values (MD, HD), tendency for ↑ ♂ platelets (HD), ↓ serum glucose (all doses), ♂ grossly enlarged urinary bladder (all doses).

* plus additional mice for toxicokinetic measurements

Toxicokinetics

Plasma $AUC_{0\text{-}24\ h}$ (µg.h/mL) - ♂/♀:

Study	Sampling week	No./sex/dose/ sample time	Sampling times#	Dose (mg/kg/day)
1578	13	3	0, 2, 4, 8, 12, 24 h	s47 [redacted]
1556	2	5	0, 2, 4, 8, 12, 18, 24 h	
SP1595*	4	3	0, 2, 4, 8, 12, 24 h	

after initiation of the dark cycle * supplementary dietary toxicokinetic study in B6C3F1 mice; actual mean drug intakes were close to nominal doses & actual mean ♂/♀ intakes, correspond to 1%, 3%, 5% dietary concentrations

8.2 Rats

Pivotal study:

Study No. and date: **AA1994** (report 745-02683), Feb. 1995-Mar. 1996
Laboratory: Parke-Davis, Ann Arbor, MI, USA
GLP compliance: Yes

Dosing route, frequency and duration: dietary x 26-52 weeks*
Strain and group size: Wistar, 25/sex*

Nominal dose (mg/kg/day)**:	s47 [redacted]	Findings ♂/♀#		
Mortality (drug-related)	NA	-	-	-
Clinical signs: tail lesions[1]	NA	-	+	++
urine staining[2]	NA	+	+	+/++
BWG[3]	NA	-	-	↓24/-
Ophthalmology	NA	-	-	-
Haematology: erythrocytes	NA	↑9/-	↑11/6	↑9/6
platelets	NA	↓18/-	↓34/19	↓36/14
platelet volume	NA	-	↑7/-	↑11/-
lymphocytes[4]	NA	-/↓26	↓32/42	↓23/36
↑ large/giant platelets (blood smears)	NA	-	+/+	+/+
Myelograms: total nucleated cells	NA	-	↓34/-	↓44/-
myeloid/erythroid ratio	NA	-	-	-
megakaryocytes	NA	-	-	-
Serum chemistry	NA	-	-	-
Urinalysis[5]	NA	-	-	-
Organ weights (body weight-relative): salivary glands	NA	-/↓17	↓19/21	↓13/18
Gross pathology	NA	-	-	-
Histopathology[6]: femoral marrow hypocellular (min.-mild)	-	-	7/3	3/5
spleen extramedullary haematopoiesis (min.-mild)	-/1	NE	NE	2/3
submandibular salivary gland secretory depletion (min.)	-	NE	-	6/3
lung foamy alveolar macrophages (min.-mild)	3/1	1/1	4/-	7/5
urinary bladder lamina propria haemorrhage (min.-mod.)	-	NE	-	5/-

* rats scheduled to be killed after 26 weeks (10/sex/group) and 52 weeks (15/sex/group); initial group size was 35/sex, with 10/sex/group being killed after 13 weeks and results reported separately (see *other studies (dietary)*)
** actual intakes were similar to nominal values
# quantitative values are *terminal* % changes from vehicle controls or incidences
- no changes or findings, NA not applicable, NE not examined or only selected rats examined
[1] erythema/sores over weeks 1-4 [2] persistent in 7 HD ♀ (>10 weeks duration)
[3] ↓ for HD ♂ associated with ↓ food consumption [4] ↓ monocytes and eosinophils of similar magnitude in MD and HD ♂ [5] urine volume not recorded (also in other dietary studies)
[6] routine examinations confined to control and HD rats, plus incidental deaths and gross findings

Erythrocyte and platelet number changes of similar magnitude were also seen at the interim (26 week) kill, at which time reduced relative salivary gland weights and histological findings (excluding spleen) were apparent. A mammary gland adenoma was identified at histological examination in 1/15 HD females in week 52. Blood smears were said to have shown spherocytes/schistocytes in 1 control, 1 LD, 1 MD and 3 HD male rats at 52 weeks (2 control and 1 HD males were affected at 26 weeks).

Other studies (dietary):

Study details	Strain and group size	Duration (wk)	Dose (mg/kg/day)#	Findings
SN: **AA1994** Date: Feb.-June 1995. Laboratory: Parke-Davis, Ann Arbor, MI, USA. GLP: Yes.	Wistar, 10/sex*.	13	s47 (MD1), s47 (MD2), s47	*Similar to the pivotal study*: clinical signs (urine staining, tail lesions), ↓ BWG (by *ca* 40% in HD ♂ and ♀), ↓ food consumption, ↑ erythrocytes, ↓ platelets, ↓ bone marrow total nucleated cells, M:E cell ratios unaffected by treatment, histological findings in urinary bladder (lamina propria oedema/haemorrhage), bone marrow, lungs, salivary glands. *Additional findings*: testicular spermatogenic epithelial degeneration (3/10 HD, min.-mild), urinary bladder mucosal epithelial hyperplasia (2/sex HD, minimal), thymic lymphoid depletion (≥MD1), pancreatic ↓ acinar cell granules (≥MD2).
SN: **1566-A** Date: Sept.-Nov 1994. Laboratory: Parke-Davis, Mississauga, Ontario, Canada. GLP: Yes.	Wistar, 15/sex[5].	4	s47	*Similar to the pivotal study*: tail lesions (dermatopathy, erythema), ↓ platelets and ↑ platelet volume (reversible). *Additional findings*[4]: ↓ relative ♂ pancreas and prostate weights (reversible).
SN: **1554** Date: Jan.-Mar. 1994 Laboratory: Parke-Davis, Mississauga, Ontario, Canada. GLP: Yes.	Wistar, 15/sex[5].	4	s47	*Similar to the pivotal study:* tail lesions (erythema, dermatopathy, necrosis), urine staining, ↓ BWG (both sexes, by 18-70%), ↓ food consumption, ↑ erythrocytes (also ♂ haematocrit, haemoglobin), ↓ platelets, ↑ platelet volume, sternal bone marrow (control, HD only examined) M:E ratio unchanged (haematological changes reversible), histological changes in lungs (foamy alveolar macrophages). *Additional findings*: initial (week 1) CNS-related clinical signs (ataxia, hypoactivity, hyperactivity), mortalities (3 HD killed), reversible ↓ sternal bone marrow megakaryocyte number, reversible ↓ serum chloride, histological findings in epididymides (luminal debris, fibrosis, hypospermia, mononuclear cell infiltration), pancreas (↓ acinus granules, single cell necrosis), thymus (involution), premature deaths showed urinary bladder gross dilation, and marked haemorrhagic necrosis or marked epithelial hyperplasia/pyelonephritis; Selected hepatic microsomal enzymes were also measured (see section 6.4).

Other studies (dietary) - continued:

Study details	Strain and group size	Duration (wk)	Dose (mg/kg/day)[#]	Findings
SN: **1537** Date: May-July. 1993 Laboratory: Parke-Davis, Mississauga, Ontario, Canada. GLP: Yes.	Wistar, 5/sex[@]	2	s47 (dietary); s47 (PO, s47	*Major findings* and *differences between dietary and gavage administration*: mortality (1 ♀ HD gavage), initial hypoactivity and ataxia, urine staining, tail dermatopathy and necrosis (diet only), ↓ BWG, ↓ HD food consumption, ↑ erythrocytes, ↓ platelets, histological findings in lungs (foamy alveolar macrophages), epididymides (inflammatory cell infiltration, HD diet only); premature death findings included marked pyelonephritis, marked urinary bladder mucosal necrosis.

[#] actual intakes were close to nominal values
[*] an additional 12/sex HD rats used only for toxicokinetic measurements; this HD not used in the pivotal 26-52 week phase
[$] 5/sex/group kept for a 4 week recovery period after cessation of dosing; an additional 3/sex (control) or 6/sex/group used only for toxicokinetic measurements
[&] limited tissue range subject to histological examination
[@] plus an additional 15/sex/group for toxicokinetic measurements

Other studies (PO):

Study details	Strain and group size	Duration (wks)	Dose (mg/kg/day)*	Findings
SN: **AA2518** Date: Oct.-Dec. 1999. Laboratory: Pfizer, Ann Arbor, MI, USA. GLP: Yes.	7 day old Wistar, 16/sex[&].	7	s47	*Similar to the pivotal (adult dietary) study*: urine staining, ↓ BWG (all doses, by 9-14%), ↓ food consumption (MD and HD, on days 15-29), no ophthalmic, urinalysis effects of treatment, slightly ↑ platelet volume (HD, by *ca* 7%). *Additional findings*[#]: clinical signs (hyperactivity, bruxism, rough pelage, salivation), ↓ serum glucose (all doses, by *ca* 20% but within reference range), no effects of treatment on bone marrow or relative organ weight values, treatment-related histological findings only in liver (minimal ↑ hepatocyte mitosis, 7/15 HD ♂ vs 3/15 controls), hepatic microsomal protein, cyt. P450 (nmol/mg protein) unaffected by treatment.

s47

[&] plus additional rats for toxicokinetic measurements
[#] there were 1 control, 6 LD, 2 MD, 3 HD premature deaths, related to apparent misdosing or of unknown cause; one HD case showed mild pyelonephritis/cystitis and urinary calculi

IV studies were carried out to support the possible use of this administration route, although according to the nonclinical expert, this is no longer being considered.

Other studies (IV):

Study details	Strain and group size	Duration (wks)	Dose (mg/kg/day)	Findings
SN: **SP1645** Date: Aug.-Oct. 1998 Laboratory: Parke-Davis, Mississauga, Ontario, Canada. GLP: Yes.	Wistar, 10/sex.	2	s47 [redacted] mg/kg/h by continuous infusion.	no drug-related mortalities, urine staining, CNS-related clinical signs (♀ sporadic hypoactivity, ♂ catalepsy), ↑ erythrocytes, ↓ ♂ platelets, ↑ platelet volume, ↓ ♂ lymphocytes, ↑ ♀ bone marrow M:E ratio (HD), ↓ serum chloride, histological findings (HD) in the lungs (foamy alveolar macrophages), ♀ urinary bladder (muscularis degeneration, 2 HD ♀), Harderian gland (↑ acinus porphyrin deposition). *Toxicokinetics*: sex-combined plasma concentrations were s47 [redacted] µg/mL (day 14) or s47 [redacted] µg/mL (4 h). *Pilot study (1636)*: same doses tested over 96 h; additional findings were HD ataxia, terminal brain (cerebellum) concentrations of s47 [redacted] µg/g vs s47 [redacted] µg/mL for plasma.
SN: **SP1637** Date: May-June 1998. Laboratory: Parke-Davis, Mississauga, Ontario, Canada. GLP: Yes.	Wistar, 10/sex*.	4	s47 [redacted] (bolus injection).	no premature deaths, CNS-related clinical signs (ataxia, hyperactivity with all doses), ↑ ♀ BWG and food consumption, ↑ ♀ bone marrow M:E ratio and ↓ ♀ total nucleated cells (MD, HD), no drug-related histological findings. *Pilot study (1632)*: 50, 150, 300 mg/kg/day x 7 days tested; no additional findings except for slightly ↓ platelets; mean terminal $AUC_{0\text{-}24\ h}$ values were 158, 467, 977 µg.h/mL.

* plus additional rats (9/sex/group) for toxicokinetic measurements

Toxicokinetics

Plasma $AUC_{0\text{-}24\ h}$ (µg.h/mL) - ♂/♀:

Study (route)	Sampling week	No./sex/dose /sample time	Sampling times	**Dose** (mg/kg/day)
1994 (diet)	13	3	0, 4, 7, 12 h# *	s47 [redacted]
1566-A (diet)	4	3	0, 4, 7, 12 h#	
1554 (diet)	4	3	0, 4, 7, 12 h#	
1537 (diet, gavage)	2	5	0, 1, 4, 7, 12, 24 h (diet)# 0, 2, 4, 7, 12, 24 h (gavage).	

Plasma $AUC_{0\text{-}24\ h}$ (µg.h/mL) - ♂/♀ (continued):

Study (route)	Sampling week	No./sex/dose /sample time	Sampling times	Dose (mg/kg/day)
AA2518 (gavage)	7	3-4	0, 4, 7, 12 h (t_{max} = 4 h)	s47 [redacted]
SP1637 (IV)	4	3	0, 0.5, 2, 6, 24 h	

* 12 h concentrations (excluding HD) in week 48 were s47 [redacted] µg/mL (♂) or s47 [redacted] µg/mL (♀)
# after initiation of the dark cycle

Plasma AUC values in study **AA2518** were considered to be underestimated, based on comparison with data in a 3 week toxicokinetic study (**AA2712**, section 11) that included an additional sample time at 1 h (at which peak concentrations were measured).
Brain (cerebrum) concentrations were measured at 2, 6 and 24 h post-injection in study **SP1637**. Mean concentrations (3/sex/dose/sample time) were highest at 2 h, respectively 5.5, 11.7 and 32.8 µg/g (brain/plasma ratios of 0.20-0.23; 0.38-0.47 at 6 h) and pregabalin was not measurable at 24 h. Plasma $t_{1/2}$ values were *ca* 2.5 h, while clearances were 205, 234 and 246 mL/h/kg and V_{ss} values were 0.57, 0.64 and 0.68 L/kg.

8.3 Cynomolgus monkeys

Pivotal study:

Study No. and date: **1992** (report 745-02646), April 1995-July 1996
Laboratory: Parke-Davis, Ann Arbor, MI, USA
GLP compliance: Yes (but not signed by study director)

Dosing route, frequency and duration: PO daily x 65-69 weeks
No./Sex/Group: 3*
Vehicle: s47 [redacted]

Dose (mg/kg/day):	0	Findings ♂/♀# s47 [redacted]			
Mortality (drug-related)	NA	-	-	-	-[m]
Clinical signs: tail dermatopathy[d]	-	-/1	2/-	-/2	3/2
soft faeces/diarrhoea	NA	-	-	-	+
BWG	NA	-	-	-	-
Ophthalmology	NA	-	-	-	-
Body and SC tail temperatures	NA	-	-	-	-
ECG (quantitative values)	NA	-	-	-	-
ECG - ambulatory (Holter)	NA	-	-	-	-
Echocardiography[e]	NA	-	-	-	-
Blood pressure	NA	-	-	-	-
Haematology	NA	-	-	-	-
Blood smear erythrocyte agglutination[n]	-	1/-	1/-	2/3	2/3
Coagulation: PT/APTT[c]	NA	-	-	-	-
bleeding times	NA	-	-	-	-
ex vivo platelet aggregation	NA	-	-	-	-/↑125[p]
Myelograms (costal)[f]	NA	-	-	-	-
Serum chemistry	NA	-	-	-	-
Urinalysis[u]	NA	-	-	-	-
Semen quality and testes volume	NA	-	-	-	-
Organ weights (body weight-relative)	NA	-	-	-	-
Gross pathology	NA	-	-	-	-
Histopathology	NA	-	-	-	-

* a further 4/sex control, 3/sex treated with 10, 25 and 100 mg/kg/day and 4/sex treated with 500 mg/kg/day were killed after 13 weeks, and results were reported separately (see *Other studies (PO)*)
[$] dose ↑ from 250 to 500 mg/kg/day from week 13
[#] quantitative values are *terminal* % changes from vehicle controls or incidences
- no changes or findings, NA not applicable, NE not examined
[im] 2 apparently incidental ♀ premature deaths (see text)
[d] erosive lesions, skin sloughing, crusts, necrosis, generally resolved by study end
[e] carried out on anaesthetised animals
[a] week 35, all slight at room temperature
[c] prothrombin and activated partial thromboplastin times
[p] ristocetin- and arachidonic acid-induced, collagen- and ADP-induced responses unaffected (citrated whole blood)
[f] flow cytometry method allowed megakaryocyte enumeration (not by a different method used at interim sacrifice)
[u] urine volumes not measured (also applies to the other studies)

The 2 HD female premature deaths showed gastric dilation/rupture with secondary septic peritonitis (found dead in week 39) or abdominal distension associated with gaseous colonic dilation and mucous-filled large intestines (killed moribund in week 65). Both deaths occurred during or shortly after the ambulatory ECG procedure, and although a relationship to treatment was not clear, the study considered these to be incidental and exacerbated by prior anaesthesia for the attachment of ECG equipment. Acute gastric dilation is known to occur spontaneously in non-human primates.

Several special investigations were incorporated into the study, including extensive cardiac measurements at 2-4 h post-dosing, as well as serum LDH and CPK isoform analysis, at intervals throughout the study. Male serum testosterone was measured at intervals as well as semen assessment (sperm count, motility, morphology) in weeks 13, 40 and 65. Coagulation investigations included terminal *ex vivo* platelet function (aggregation and ATP release by collagen, ADP, ristocetin, arachidonic acid). SC tail temperature measurements were included to investigate the pathogenesis of tail dermatopathy, as well as examination of blood smears for erythrocyte agglutination at room temperature.

Other studies (PO):

Study details	Group size	Dose (mg/kg/day)[$] and duration	Findings
SN: **1992** (report 745-02559)[#]. Date: April-July 1995. Laboratory: Parke-Davis, Ann Arbor, MI, USA. GLP: Yes.	3-4/sex.	s47 [redacted] s47 [redacted] x 13 weeks.	*Similar to the pivotal study*[6]: tail dermatopathy s47 [redacted] mg/kg/day; 2 HD required distal tail amputation), soft faeces/diarrhoea (HD). *Additional findings*: histological correlates of dermatopathy (epidermal ulceration, dermal neutrophilic inflammation), enlarged/vacuolated bone marrow megakaryocytes (1 HD ♂).
SN: **1929** (report 745-02329). Date: April-Dec. 1994. Laboratory: Parke-Davis, Ann Arbor, MI, USA. GLP: Yes.	4/sex**.	s47 [redacted] x 4 weeks. s47 [redacted] x 4 weeks	*Similar to the pivotal study*: tail sores, soft faeces/diarrhoea. *Additional findings*: CNS-related clinical signs (ataxia, hypoactivity), premature deaths of unknown cause (1♂/2♀ HD, plus 1 ♂ HD killed because of severe tail lesions), ↑ BW-relative heart weights in individual animals, gross ♀ cardiac enlargement or ventricular hypertrophy, sporadic histological findings in the heart (intraventricular septum/ventricle degeneration/necrosis/fibrosis) and testes/epididymides (hypospermia) at s47 [redacted] s47 [redacted] mg/kg/day.

Other studies (PO) -continued:

Study details	Group size	Dose (mg/kg/day)[$] and duration	Findings
SN: **1533** (report 250-01720). Date: Feb. 1994. Laboratory: Parke-Davis, Mississauga, Canada. GLP: Yes.	4/sex.	s47 [redacted] x 4 weeks (scheduled).	Study terminated after the first dose because of premature deaths of unknown cause (2 MD, 2 HD); clinical signs were ataxia/hypoactivity (MD, HD) and stereotypic behaviour (HD).
SN: **1544** (report 250-01713). Date: Aug. 1993-Jan. 1994. Laboratory: Parke-Davis, Mississauga, Canada. GLP: Yes.	2/sex (1/sex controls).	s47 [redacted] x 2 weeks; HD was an additional group subsequently investigated after the main study with animals not being killed.	premature death (♀ at 1000 mg/kg/day), soft faeces/diarrhoea, blood-tinged nasal discharge, ↓ erythrocytes, ↑ neutrophils, gross tail sores, no drug-related histological findings.
SN: **1916** (report 750-02268). Date: Mar.-April 1994 Laboratory: Parke-Davis, Ann Arbor, MI, USA. GLP: Yes.	2/sex.	s47 [redacted] x 4 days.	premature deaths of unknown cause (2/sex at s47 [redacted] mg/kg/day, qd); ataxia/hypoactivity s47 [redacted] mg/kg/day, qd and given bid), soft faeces/diarrhoea (all doses); serum biochemistry/haematology measurements excluded, only premature deaths necropsied (this was primarily a clinical tolerance and toxicokinetic study).

s47 [redacted]

[#] interim phase of pivotal study
* animals not killed at 13 weeks; dose ↑ to 500 mg/kg/day and treatment continued (see *Pivotal study*)
[&] investigations included ambulatory ECG (as well as resting ECG), echocardiography, blood pressure, body and SC tail temperatures, semen analysis
** 1/sex/group kept for a 4 week recovery period after cessation of dosing
[@] given as 2 divided doses *ca* 4 h apart

Clinical cardiac data and heart histological findings in the 13 week interim phase of study **1992** were reviewed (report **745-02345**) prior to release of the final study report, in view of the findings in the 4 week study. A retrospective assessment of peripheral blood erythrocyte and platelet morphology, and bone marrow composition, in study **1992** was carried out (report **745-03746**) by examination of blood smears, and histological sections and cytocentrifuge preparations of femoral marrow. The only identified effects of treatment were slightly lower numbers of megakaryocytes in terminal HD males and females (by 24-32%, number/5000 cells in femoral sections) and slightly higher terminal M:E ratios in females treated with s47 [redacted] mg/kg/day (cytocentrifuge preparations).

Other studies (IV):

Study details	Group size	Dose (mg/kg/day) and duration	Findings
SN: **SP1644** (report 250-01817). Date: Aug.-Sept. 1998. Laboratory: Parke-Davis, Mississauga, Canada. GLP: Yes.	3/sex.	s47 (mg/kg/h) x 2 weeks (continuous infusion).	♀ premature deaths (1 HD died; 1 MD, 1 HD killed), clinical signs of ataxia, hypoactivity, dermatopathy (tail and other areas), widespread swelling, red nasal discharge, ↓ platelets, sporadic hyperglycaemia and glucosuria (different animals affected), multiple (apparently secondary) serum chemistry changes in some animals, histological findings in skin (widespread dermal/SC vasculopathy, vascular necrosis with associated epidermal degeneration/ulceration, extensive SC oedema) and nasoturbinates (mucosal degeneration, attenuation, ulceration with haemorrhagic exudate). *Toxicokinetics*: sex-combined day 14 plasma concentrations were 17.4, 30.6, 50.9 µg/mL; corresponding CSF values were 1.0, 2.3, 3.4 µg/mL and brain values were 1.9, 4.1, 6.4 µg/g. *Pilot study (1634)*: doses (mg/kg/h) of 2 x 96 h, 4 x 96-h, 6 x 72 h, 8 x 24 h tested using 1/sex in a cross-over design with no additional findings; there was 1 HD premature death.
SN: **2314** (report 745-03033). Date: May-June 1998. Laboratory: Parke-Davis, Ann Arbor, MI, USA. GLP: Yes.	3/sex.	s47 x 4 weeks (1-10 min injections).	CNS-related clinical signs (convulsions (MD, HD), ataxia, tremors (HD)), HD ♂ nasal discharge, ↓ HD ♂ erythroid values, ↑ HD ♀ bone marrow M:E ratio (due to ↑ E component), no drug-related effects on BWG, ophthalmology (no data), urinalysis, organ weights, gross pathology and histopathology, ECG examinations not carried out.

Toxicokinetics

Plasma concentrations (µg/mL) or $AUC_{0\text{-}24\ h}$ (µg.h/mL) - ♂/♀

Study (route)	Sampling week	No./dose/ sample time	Sampling times	**Dose** (mg/kg/day)
1992 (PO)	13	3-6/sex	predose 2 h	s47 [redacted]
	65	2-3/sex	predose 2 h	
1929 (PO)	day 1 week 4	4/sex	0, 1, 2, 4, 6, 8, 12, 24 h	
1544 (PO)	week 2	2/sex	0, 1, 2, 7, 12 h	
1916 (PO)	day 2 day 4	2/sex	0, 2, 4, 6, 12, 24 h (plus 1, 8 h with bid dosing).	

Plasma concentrations (µg/mL) or $AUC_{0\text{-}24\ h}$ (µg.h/mL) - ♂/♀ (continued):

Study (route)	Sampling week	No./dose/ sample time	Sampling times	Dose s47 [redacted]
2314 (IV)[&]	day 1 week 4	3/sex	0, 0.5, 2, 7, 24 h	s47 [redacted]

* 3 ♂/2 ♀ in week 4
s47 [redacted]/kg given bid *ca* 4 h apart
[&] overall mean (dose and sex) $t_{1/2}$ value of 5.3 h

9.0 GENOTOXICITY

Test type and details	Test system	Test conditions*	Results	Validity
Bacterial reverse gene mutation. SN: **1780**. Date: July-Aug. 1992 Laboratory: Parke-Davis, Ann Arbor, MI, USA. GLP: Yes.	*S. typhimurium*, strains TA 100, 98, 1535, 1537, 1538.	Concentrations of s47 [redacted] s47 [redacted] µg/plate tested, with and without S9 activation, in 2 separate plate incorporation assays; single preincubation assay (+S9) also carried out.	**Negative** no indications of bacterial toxicity.	↑ revertant colonies with positive controls.
Bacterial reverse gene mutation. SN: **AA2670**. Date: May-June 2000. Laboratory: Pfizer, Ann Arbor, MI, USA. GLP: Yes.	*S. typhimurium*, strains TA 98, 100, 1535, 1537.	Concentrations of s47 [redacted] s47 [redacted] µg/plate tested, with and without S9 activation, in 2 separate plate incorporation assays; single preincubation assay (+S9) also carried out.	**Negative** no indications of bacterial toxicity.	↑ revertant colonies with positive controls.
Bacterial reverse gene mutation. SN: **2477**. Date: June 1999. Laboratory: s47 [redacted] GLP: Yes.	*E. coli* WP2*uvr*A.	Concentrations of s47 [redacted] µg/plate tested, with and without S9 activation, in 2 separate experiments.	**Negative** no indications of bacterial toxicity.	↑ revertant colonies with positive controls.
Bacterial reverse gene mutation. SN: **AA2734**. Date: Jan.-Aug. 2001. Laboratory: Pfizer, Ann Arbor, MI, USA. GLP: Yes.	*S. typhimurium*, strains TA 98, 100, 1535, 1537; *E. coli* WP2*uvr*A.	Concentrations of s47 [redacted] µg/plate tested in the presence of S9 activation (hepatic preparations from Aroclor 1254-treated B6C3F1 and CD-1 mice), in 2 separate experiments/S9 preparation.	**Negative** no indications of bacterial toxicity.	↑ revertant colonies with positive controls.

Document 1

Continued:

Test type and details	Test system	Test conditions*	Results	Validity
Mammalian cell gene mutation (*hgprt* locus). SN: **1950**. Date: July-Sept. 1994. Laboratory: Parke-Davis, Ann Arbor, MI, USA. GLP: Yes.	Chinese hamster ovary (CHO) cells *in vitro*.	Cells incubated with s47 s47 g/mL x 3 h, with and without S9 activation, and after an 8 day expression period were plated in medium containing 6-thioguanine for mutant colony enumeration (2 separate experiments); water vehicle control included; plating efficiency (PE) determined after treatment and after the expression period.	**Negative** PE unaffected by treatment.	↑ mutant colony frequencies with positive controls.
Chromosome aberration assay. SN: **1940**. Date: Aug.-Dec. 1994. Laboratory: Parke-Davis, Ann Arbor, MI, USA. GLP: Yes.	CHO cells *in vitro*.	Cells incubated with s47 µg/mL x 3 h, with and without S9 activation, and demecolcine-arrested metaphases harvested at 20 h (200/concentration scored for chromosomal aberrations (CA)); repeat experiment carried out with 20 h exposure without S9 activation; cell growth (CG), post-treatment plating efficiency (PE), proliferation index (PI, assessed in cytochalasin B-treated cells) also measured.	**Negative** (see text below). high-concentration PE ↓ by 25% (3 h +S9) or 72% (20 h -S9) and PI ↓ by 46% (20 h -S9).	↑ cells with CA and ↑ no. CA/cell with positive controls.
Unscheduled DNA synthesis. SN: **1889**. Date: Oct.-Nov. 1993. Laboratory: Parke-Davis, Ann Arbor, MI, USA. GLP: Yes.	♂ Wistar rats.	Rats (3-4/group) treated PO with doses of s47 mg/kg and killed at 2 h and 16 h; hepatocytes incubated with ^{3}H-thymidine x 4 h followed by 14-18 h with unlabelled thymidine; net nuclear grains (NG) determined by autoradiography (100 cells/rat scored); vehicle control rats killed at 16 h, positive control rats (10 mg/kg dimethyl-nitrosamine) killed at 2 h.	**Negative**	↑ NG and % of cells in repair (NG ≥5) with positive control.
Unscheduled DNA synthesis. SN: **AA2792**. Date: June-Sept. 2001. Laboratory: s47 Laboratories, s47 s47 GLP: Yes (report not signed by study director or QA unit).	♀ B6C3F1 and CD-1 mice.	Mice treated PO with doses of s47 mg/kg and killed at 2-4 h and 14-16 h; hepatocytes incubated with ^{3}H-thymidine x 4 h followed by overnight incubation with unlabelled thymidine; NG determined by autoradiography (100 cells/mouse scored, at least 3 mice/group); positive control mice killed at s47 mg/kg dimethylnitrosamine) or 14-16 h (200 mg/kg fast garnet GBC).	**Negative** (both strains).	↑ NG and % of cells in repair (NG ≥5) with positive control.

Continued:

Test type and details	Test system	Test conditions*	Results	Validity
Micronucleus test. SN: **1945** Date: Aug. 1994. Laboratory: Parke-Davis, Ann Arbor, MI, USA. GLP: Yes.	Wistar rats.	Single PO doses of s47 s47 mg/kg; femoral bone marrow collected from 5/sex/dose at 24 h and 48 h for measurement of micronucleated (mn) PCE (2000/rat scored), % PCE (400 erythrocytes/rat scored); vehicle controls (5/sex) positive controls s47 /kg IP cyclophosphamide, 5/sex) killed at 24 h only.	**Negative** no ↑ in mnPCE; 24 h % PCE ↓ slightly but within historical control values.	↑ mnPCE with the positive control. Clinical signs: transient ataxia/ hypoactivity
Micronucleus test. SN: **AA2657** Date: Jan.-Feb. 2001. Laboratory: Pfizer, Ann Arbor, MI, USA. GLP: Yes.	B6C3F1, CD-1 mice.	Single PO doses of s47 s47 kg; femoral bone marrow collected from 5/sex/dose/strain at 24 h and 48 h for measurement of mnPCE and % PCE using a flow cytometry technique; vehicle controls (5/sex/strain), positive controls (s47 mg/kg IP cyclophosphamide, 5/sex/dose/strain) killed at 24 h only.	**Negative** no ↑ mnPCE in either strain, no change in % PCE.	↑ mnPCE with positive control. Clinical signs: hypoactivity

* *in vitro* S9 activation preparation obtained from Aroclor 1254-treated rat liver (except for **AA2734**), *in vivo* PO vehicle was 0.5% methylcellulose

In study **1940**, there was a significant increase in the number cells with chromosomal aberrations at the highest concentration of s47 µg/mL in the presence of S9 (6.5% vs 2.5% for the vehicle controls and an upper historical control value of 6.3%). The criteria for a positive response included significant increases at 2 consecutive concentrations, which was not satisfied, and s47 µg/mL was equivalent to 10 mM, the highest recommended concentration for this assay. Respective numbers of cells with chromosomal aberrations at the lower concentrations tested s47 /mL) were s47

Pregabalin and N-methylpregabalin were analysed for genotoxicity (and some other toxicological) structural alerts using s47 software (report **745-03407**). A number of naturally occurring and synthetic compounds with structural similarities to pregabalin were also included. s47 analysis flagged pregabalin and N-methylpregabalin for forestomach irritancy and carcinogenicity, based on the presence of a short chain aliphatic acid (plus a secondary amine in the case of the metabolite), a result which was also obtained with GABA and ε-amino-n-caproic acid. Pregabalin was negative in the s47 analysis, while N-methylpregabalin was flagged as a male mouse carcinogen, a result also obtained with histamine, lysine, ornithine and lysine. Overall, it was considered that no relevant structural alerts were identified.

10. CARCINOGENICITY

10.1 Long-term studies

Tumour incidences were routinely analysed using the 2-tailed Peto (time-adjusted) trend test, with significance at the 5% level for rare tumours (control incidence <1%) and at the 1% level for common tumours. A 1-tailed exact positive trend test was used where total numbers of tumours was small (≤12), with respective significance at the 2.5% and 0.5% levels. Pairwise comparisons in the mouse studies were by Fischer's exact test.

10.1.1 Mice

Study No. and date: **AA2236** (report 745-03275), Sept. 1997-Oct. 1999
Laboratory: s47 [redacted]
GLP compliance: Yes

Dosing route, frequency and duration: dietary x 104 weeks
Strain and group size: B6C3F1, 64-66/sex

Dose (mg/kg/day)*:	0	s47 [redacted]		
	Findings ♂/♀#			
Mortality (number)	8/12	12/19	24/33	42/38
Clinical signs: abdominal internal mass	21/3	19/9	44/7	56/42
BWG[f]	NA	↑33/37	↑53/62	↑33/37
Ophthalmology	NA	-	-	-
Haematology: platelets	NA	-/↑36	↑35/32	↑33/58
Organ weights (BW-relative): testes	NA	↓17	↓23	↓32
liver	NA	-	-/↑13	-/↑19
Tumour ↑ mouse incidence: haemangiosarcoma[l]	2/2	3/7	19/19	22/25
Non-neoplastic findings: testicular tubular degeneration[d]	-	2	2	7
spleen ↑ extramedullary haematopoiesis	11/11	14/18	24/18	21/27
urinary bladder dilation	1/-	2/-	2/-	15/-
bone marrow megakaryocytic hypercellularity[r]	15/12	25/17	39/27	46/33

terminal mouse incidences or % change from control values
* mean actual intakes were close to nominal values
[f] associated with ↑ food consumption
NA = not applicable, - = no change or not present
[l] multiple sites, mainly liver, spleen, bone marrow
[d] bilateral, trace-moderate
[r] identified in a further retrospective evaluation of non-neoplastic findings in selected tissues (report **745-03454**)

Mouse incidences of haemangiosarcomas were significant for trend at the 1% level for common tumours in both sexes, while there were no significant pairwise differences between control and LD incidences. Significant trends for other tumours related to those with only single HD cases, and female mammary gland adenomas (1 control and 3 HD cases) which were not significant with the exact trend test.

The retrospective evaluation (by Pfizer) also identified increased incidences of liver sinusoidal cell hyperplasia and bile stasis (LD, MD), and coagulative necrosis (MD, HD), together with lung foamy macrophages and granulomatous inflammation (HD). The bone marrow megakaryocytic hyperplasia was graded as minimal-moderate, with 2 LD, 10 MD and 14 HD cases being moderate. Quantitative measurement of megakaryocytes in femoral bone marrow sections (number/5000 haematopoietic cells) was carried out in another retrospective evaluation (report **745-03456**). Dose-related increases in megakaryoblasts (up to 5-6x) and early and late megakaryocytes (up to *ca* 2x) were observed, which contrasted with results for rats, which were assessed at the same time. Increases were not seen in rats from a carcinogenicity study (**AA2235**,

section 10.1.2), and total megakaryocytes were significantly reduced in the HD groups (by 12% in males and 24% in females).

Study No. and date: **AA2658**, April 2000-April 2002 Laboratory: s47 [redacted] GLP compliance: Yes				
Dosing route, frequency and duration: dietary x 104 weeks Strain and group size: CD-1, 65/sex	Findings ♂/♀#			
Dose (mg/kg/day)*:	0	200	1000	5000
Mortality (number)	32/37	34/35	38/38	37/44[m]
Clinical signs: swollen abdomen	5/17	8/18	22/29	21/25
UG staining	-/2	1/1	5/7	6/2
Ophthalmology	NA	-	-	-
BWG[f]	NA	↑55/30	↑67/54	↑43/32
Haematology: platelets	NA	-	-	↑32/x
platelet volume	NA	↑3/-	↑5/3	↑5/x
erythrocytes	NA	-	-	↓9/x
erythrocyte volume	NA	-	↑5/-	↑8/x
Organ weights (BW-relative): testes	NA	-	↓16	↓16[w]
liver	NA	-	-/↑39	-/↑17
Tumour ↑ mouse incidence: haemangiosarcoma[h]	2/6	5/9	6/10	14/13
Non-neoplastic findings: lung alveolar macrophages	14/15	10/15	6/14	16/50[l]

[#] terminal mouse incidences or % change from control values
* mean actual intakes were close to nominal values (2.2-3.6% lower)
NA = not applicable, - = no change or not present, x = no concurrent controls
[m] ♀ killed at week 100 because of higher mortality
[f] associated with ↑ food consumption
[h] multiple sites, mainly liver, spleen, uterus
[l] often associated with ↑ incidences of perivascular lymphoid infiltration, cholesterol clefts
[w] HD prostate value ↓ by 17%

A one-tailed Peto trend test was used for tumour incidence analysis, with significance set at 2.5% (rare tumours) or 0.5% (common tumours). Haemangiosarcoma incidences showed a significant positive trend in males, while group comparison with the controls showed a significant difference only for the HD group. A borderline significance was seen for positive trend in females (p=0.0058), and this appeared to be a biologically significant effect.

A retrospective evaluation of femoral bone marrow sections, including quantitative measurement of megakaryocytes (number/5000 haematopoietic cells) showed some effects of treatment (report **745-03692**). Total counts (megakaryoblasts, early and late megakaryocytes, megakaryocytic nuclei or cytoplasm) were increased in both sexes at all doses (1.2-2.1x control values), and an incidental finding was a paucity of megakaryoblasts in this strain compared with B6C3F1 (30-fold difference in control females). Additional findings were a drug-related decrease in the incidence of myeloid hyperplasia and increases in incidences of mitotic megakaryocytes and macrophage infiltration, and an overall conversion from mainly myeloid to mainly erythroid appearance. Peripheral blood platelet and erythrocyte morphology was examined in blood smears from mice in both carcinogenicity studies, in another retrospective evaluation (report **745-03714**). Dose-dependent increases in mouse incidences of variable platelet size, giant platelets and platelet aggregates, with evidence of degranulation, and schistocytes (fragmented erythrocytes) were observed in both strains, but were more pronounced in B6C3F1 mice. Respective control, LD, MD and HD schistocytosis incidences in males, for example, were 1/53, 1/47, 6/36 and 7/17 (B6C3F1) or 0/46, 2/38, 1/39 and 4/39 (CD-1).

Toxicokinetics

Plasma concentration (µg/mL) or $AUC_{0\text{-}24\,h}$ (µg.h/mL) - ♂/♀:

Study	Sampling week	No./sex/dose /sample time	Sampling times#	Dose (mg/kg/day) 200	1000	5000
AA2236*	104	5 (B6C3F1)	4 h	s47 [redacted]		
AA2658	104	5 (CD-1)	4 h			
AA2582[&]	4	4 (B6C3F1)	2, 4, 6, 8, 12, 24 h			
AA2794[&@]	4	4 (CD-1)	2, 4, 6, 8, 12, 24 h			

after initiation of the dark cycle
*6/10 control samples had measurable drug (<0.35 µg/mL except for one ♂ sample (70 µg/mL), apparently transposed from the HD group (one ♂ HD sample value was 0.25 µg/mL))
$ week 100 for HD ♀
& supplementary dietary toxicokinetic studies; t_{max} = 4-8 h
@ actual mean intakes were lower than nominal values (88-94%)

Although 4 h concentrations in the carcinogenicity studies were higher in CD-1 compared with B6C3F1 mice, this was not the case for $AUC_{0\text{-}24\,h}$ values in the 4 week toxicokinetic studies.

10.1.2 Rats

Study No. and date: **AA2235**, Sept. 1997-Oct. 1999
Laboratory: s47 [redacted]
GLP compliance: Yes

Dosing route, frequency and duration: dietary x 104 weeks
Strain and group size: Wistar, 65/sex

Dose (mg/kg/day)* - ♂/♀:	Findings ♂/♀# 0	50/100	150/300	450/900
Mortality (number)	33/30	36/17	32/12	23/20
Clinical signs: urine staining	-	-/+	-/+	-/+
Ophthalmology	NA	-	-	-
BWG[f]	NA	↑12/28	-	↓21/42
Haematology: erythrocytes	NA	↑4/9	↑9/8	↑12/14
platelets	NA	↓4/11	↓14/12	↓20/19
Organ weights (BW-relative)[w]: uterus[u]	NA	↓30	↓25	↑63
Tumour incidence (drug-related ↑)	NA	-	-	-
Non-neoplastic findings:				
testes tubular atrophy/aspermatogenesis	24	35	40	43
eye retinal atrophy	-/5	1/10	2/16	3/25
uterus dilation	8	11	8	35

terminal rat incidences or % change from control values
* mean actual intakes were close to nominal values
NA = not applicable, - = no change or not present
[f] associated with corresponding changes in food consumption
[w] changes for several organs were apparently related to differences in terminal BW, or ↓ incidences of pituitary tumours
[u] associated with HD uterine distension with or without fluid at gross examination

Analysis of tumour incidences showed significant positive trends only for male brain meningioma and female skin squamous cell carcinoma. For both types, these occurred as only 2

HD cases, and significance was not achieved using an exact trend test appropriate for small tumour numbers. Incidences of several tumour types showed significant negative trends, e.g. female mammary gland fibroadenomas (22 control, 7 LD, 5 MD, 2 HD) and pituitary adenomas (38, 27, 16, 12 corresponding cases).

Study No. and date: **AA2299**, April 1998-April 2000 Laboratory: s47 [redacted] GLP compliance: Yes				
Dosing route, frequency and duration: dietary x 104 weeks Strain and group size: Wistar, 65/sex			Findings ♂/♀[#]	
Dose (mg/kg/day)* - ♂/♀:	0	50/100	150/300	450/900
Mortality (number)	27/31	27/29	19/17	20/32
Clinical signs: UG staining	NA	-/+	-/+	-/+
Ophthalmology[o]: keratitis with neovascularisation	19/-	11/1	39/1	58/4
pale fundus	4/13	5/18	2/41	9/42
BWG[w]	NA	↑10/28	-	↓22/39
Haematology: erythrocytes[e]	NA	↑5/10	↑12/15	↑18/18
platelets	NA	↓12/18	↓19/25	↓27/29
Organ weights (BW-relative)[p]: uterus[u]	NA	-	↑49	↑57
Tumour incidence (drug-related ↑)	NA	-	-	-
Non-neoplastic findings: eye, chronic corneal inflammation	9/-	13/2	24/1	38/2
retinal atrophy	-/8	1/9	11/21	33/33
adrenal cortical angiectasis	7/60	15/61	16/61	22/55
uterus dilation	9	6	16	37
uterus inflammation[i]	4	-	1	16

[#] terminal rat incidences or % change from control values
* mean actual intakes were close to nominal values
NA = not applicable, - = no change or not present
[o] number of eyes affected; ♂ fundus difficult to examine and pale change was probably underestimated
[w] associated with sporadic ↓ in HD food consumption
[e] associated ↑ haemoglobin and haematocrit (♂ HD, ♀ all doses)
[p] ↓ in ♂ pituitary values reflect lower incidence of adenomas
[u] HD ↑ associated with uterine distension at gross examination
[i] acute, chronic, endometrial, transmural combined; possibly related to slightly ↑ HD ovarian atrophy which may be secondary to ↓ terminal BW

This was originally a backup study in which histological examinations were not scheduled, but these were subsequently carried out at another laboratory. Significant negative trends were observed for several tumour types, including pituitary pars distalis adenomas (33 control, 24 LD, 16 MD, 8 HD in males). Positive trends were noted only for granular cell tumours of the cerebrum (2 male HD cases) and heart schwannomas (1 LD and 3 HD male cases), but these were not significant by the more appropriate exact test for small tumour numbers.
Haemangiosarcomas were infrequent and incidences (0-3/group) were not treatment-related.

Toxicokinetics

Plasma concentration (µg/mL) or $AUC_{0\text{-}24\ h}$ (µg.h/mL) - ♂/♀:

Study	Sampling week	No./sex/dose /sample time	Sample times#	Dose (mg/kg/day) - ♂/♀ 50/100	150/300	450/900
AA2235	104	5	7 h	s47 [redacted]		
AA2299	104	5	7 h			
2581*	4	4	2, 4, 6, 8, 12, 24 h			

after initiation of the dark cycle * supplementary dietary toxicokinetic study in Wistar rats; actual mean ♂ drug intakes in week 4 were 13-17% above nominal doses

10.2 Short/medium-term studies

No studies.

10.3 Other studies

The following studies were related to possible mechanism(s) involved in the pathogenesis of mouse haemangiosarcomas.

In vitro and *ex vivo*:

Study or Report	Measurements/assays	Results
AA3044	effects of pregabalin s47 [redacted] µg/mL) on the 72 h proliferation of primary spleen endothelial cells (SEC) from ♀ B6C3F1 mice measured by cell counts; several different growth factor combinations used.	no effects of pregabalin.
AA3033	72 h proliferation of primary SEC from ♀ B6C3F1 mice measured by s47 [redacted] uptake; effects of pregabalin s47 [redacted] µg/mL, in the presence of s47 [redacted] FCS#) and serum (0.5%, 2.5%) from ♀ B6C3F1 mice treated with pregabalin s47 [redacted] s47 [redacted] ng/kg/day by dietary administration x 2 weeks) investigated; 20% FCS included as a positive control for enhancement.	no effects of pregabalin or treated mouse serum.
AA2608	7 day vascular outgrowth from aortic rings from ♂ B6C3F1 mice measured by s47 [redacted] s47 [redacted] effects of pregabalin (1-200 µg/mL, in the absence of FCS) investigated on naive rings (a) and rings pretreated with 20% FCS and showing a positive outgrowth response (b); FCS (2.5-20%) used as a positive control.	minimal ↑ seen with 200 µg/mL (a, *ca* 30% of the response elicited by 5% FCS), and with all doses (b, maximal at 10 µg/mL and *ca* the same response as that elicited by 2.5% FCS).
AA2826	primary cultures of SEC and bone marrow cells obtained from ♀ B6C3F1 mice were treated with up to 1000 µg/mL pregabalin (respectively for 24-48 h and 1-3 weeks) and Western blot analysis for nitric oxide synthetase (NOS) isozymes carried out.	nNOS and iNOS isozymes not detectable, eNOS in SEC unaffected by treatment, ↑ eNOS in bone marrow cells (e.g. by 150% with 1 µg/mL x 1 week) but no consistent effect of dose or exposure time apparent.

In vitro and *ex vivo* - continued:

Study or Report	Measurements/assays	Results
AA2801	platelets from ♀ B6C3F1 and CD-1 mice, Wistar rats examined for baseline function and effects of pregabalin (10-500 µg/mL); measurements included platelet counts/volume, morphology, adhesion, activation (P-selectin), aggregation, content and release of growth factors, number of reticulated platelets (baseline only); PT, APTT, fibrinogen also measured.	*effects of pregabalin*: none identified. *baseline values*: differences seen for initial adhesion (CD-1>B6C3F1 and rat), thrombopoietin and PDGF[&] content (mice>>rats), bFGF[&] content (rats>>mice), degenerative morphology changes over 2 h (rat>mice).
AA3045	effects of pregabalin (s47 µg/mL) on megakaryocyte development in bone marrow cultures from ♀ B6C3F1 mice investigated; short-term (x 5 days, supplemented with thrombopoietin and stem cell factor) and long-term (2 days - 3 weeks) assays conducted; total nucleated cells, megakaryocytes (CD41 +ve) and megakaryocyte maturation (DNA content) measured.	*short-term cultures*: no stimulation of megakaryocyte proliferation or maturation, ↓ in total nucleated cells and CD41 +ve cells at s47 µg/mL (by *ca* 35%). *long-term cultures*: no stimulation of megakaryocyte proliferation or maturation, ↓ in total nucleated cells and CD41 +ve cells at 100 and 1000 µg/mL after 3 weeks (by *ca* 37-80%).
745-03324	analysis of haemangiosarcoma and normal liver samples from carcinogenicity study **AA2236** for the presence of *Heliobacter hepaticus* DNA.	no positive reactions found.
745-03327	analysis of haemangiomas from carcinogenicity study **AA2236** for mutations in the p53 suppressor and Ha- and Ki-*ras* oncogenes.	no Ha- or Ki-*ras* mutations detected; 18 p53 gene mutations seen in 14/174 tumours mainly (16/18) guanine to adenine or thymidine to cytosine transitions; excluding 4 silent cases, 13/14 were on exon 5, in a gene sequence said to be divergent between mice and humans.
745-03739	liver, spleen, bone marrow, lung (negative control) RNA obtained from control and treated mouse samples in study **AA2795** (below) and screened for expression of wide range of genes*.	some control and test sample differences were observed, but changes were small, not consistent between target organs, and appeared not to be biologically significant.
745-03740	binding of [^{3}H]pregabalin to Wistar rat and B6C3F1 mouse bone marrow and B6C3F1 mouse spleen endothelial cell membranes measured; a positive control included s47 s47 Western blot analysis of mouse liver, spleen, brain, platelet lysates for $\alpha_2\delta$-2 and $\alpha_2\delta$-1 proteins also carried out.	no specific [^{3}H]pregabalin binding measurable; $\alpha_2\delta$-2 and $\alpha_2\delta$-1 both present in brain but not platelets; small amounts of $\alpha_2\delta$-2 also present in spleen and liver may be from contaminating smooth muscle cells.

In vitro and *ex vivo* - continued:

Study or Report	Measurements/assays	Results
745-03835	reanalysis of liver cell proliferation in samples from studies **AA2787**, **AA2795**, **AA2935** (below) using a different quantification technique, i.e. s47 s47 relative to total endothelial cells/image rather than relative to total nuclei/image; NB absolute numbers of hepatocytes>>. endothelial cells>>Kupffer cells.	**AA2787**: no changes seen at 26 weeks; ↑ at 52 weeks with s47 (MD) and s47 (HD) mg/kg/day for endothelial cells (by 136%, 152%), hepatocytes (by 270%, 180%), Kupffer cells (by 99%, 108%); ↑ were associated with low control values (vs those at 26 weeks). **AA2795**: only s47 mg/kg/day at 4 and 13 weeks tested; ↑ seen for endothelial cells at 4 weeks (by 37%) and hepatocytes at 13 weeks (by 150%, associated with a low control value). **AA2935**: only s47 mg/kg/day at 4 weeks tested; minimal ↑ Kupffer cells (by 6%).
745-03855	immunohistochemical/immunofluorescence analysis of spleen, bone marrow (femoral, sternal) and liver samples from mouse (**AA2787**, **AA2892**, **AA3012**) and rat (**AA3053**, **AA2796**) studies for VEGF and bFGF, and for Flk-1[&] in 12 month mouse and rat liver samples.	i) ↑ VEGF in spleen of B6C3F1 (but not CD-1) mice; after 12 months apparent with all doses s47 mg/kg/day); ↑ also seen with HD in sternal (but not femoral) bone marrow at 12 months; not found in liver. ii) ↑ bFGF in sternal (but not femoral) bone marrow from B6C3F1 and CD-1 mice s47 mg/kg/day x 12 months). iii) equivocal ↑ liver Flk-1 in B6C3F1 mice s47 day x 12 months); not examined in CD-1 mice. iv) no drug-related changes seen in rats.

[#] FCS = fetal calf serum

* n=122 in total, associated with cellular proliferation, control and signalling, platelet and endothelial cell function, angiogenesis, macrophages and inflammation, and extracellular matrix components

[&] PDGF = platelet-derived growth factor, VEGF = vascular endothelial growth factor, bFGF = basic fibroblast growth factor, Flk-1 = VEGF receptor 2 (one of 2 tyrosine kinase-linked receptors expressed on endothelial cells)

In vivo (short-term):

Study	Treatment* and measurements	Results
SP1733	♀ B6C3F1 mice treated with s47 mg/kg/day of pregabalin x 4 weeks and cell surface glycoprotein labelled with IV sulfo-NHS-LC-biotin; mice were then killed at 24-120 h and platelet rich plasma treated with a streptavidin-conjugated fluorescent marker; fluorescent cells enumerated by flow cytometry.	treatment had no effect on the time-dependent ↓ in fluorescent platelets.
AA2672	♂ B6C3F1 and CD-1 mice treated with s47 or s47 of pregabalin x 4 weeks and 4 days prior to necropsy were implanted with BrdU-containing osmotic pumps; liver sections assessed for cellular proliferation and apoptosis (spleen and bone marrow also collected but method was unreliable for these tissues).	treatment resulted in ↑ endothelial cell proliferation in B6C3F1 mice (by 38%) and ↓ of similar magnitude in CD-1 mice, Kupffer cell proliferation ↓ in CD-1 mice, hepatocyte proliferation unaffected by treatment, apoptosis very low and unaffected by treatment.

In vivo (short term) - continued:

Study	Treatment* and measurements	Results
AA2868	♀ B6C3F1 mice treated with s47 mg/kg/day of pregabalin and killed after 4, 7, 14 and 28 days for the measurement of liver cell proliferation as in study **AA2672**; platelet and plasma VEGF and PDGF#, platelet counts, bone marrow eNOS (Western blot analysis) also measured.	↑ endothelial cell proliferation by 90%, 21%, 28%, 15% in treated group at the 4 sample times (significant on day 4 and 14) but the day 4 control value was particularly low, ↑ hepatocyte proliferation in the treated group but variable control values and no clear temporal effect, Kupffer cell proliferation unaffected by treatment, no consistent changes in growth factors or platelet numbers, eNOS not detectable.
AA2795	♀ B6C3F1 mice treated with s47 mg/kg/day of pregabalin and were killed after 1 or 3 months; measurements included platelet morphology and activation/aggregation, platelet and plasma growth factors, bone marrow megakaryocyte counts (manual), liver cell proliferation as in study **AA2672**.	numerous effects of treatment were identified, including: i) slightly ↑ erythroid values and platelet volume, abnormal platelet morphology in blood smears (↑ non-uniform size, presence of giant platelets, hypogranularity, presence of aggregates). ii) ↓ ADP-induced aggregation and abnormal responses (disaggregation after initial aggregation), ↑ platelet basal activation. iii) ↑ bone marrow megakaryocytes (by *ca* 50% at both sample times), ↓ M:E ratio (by 30-36%, reflecting ↑ erythroid elements) iv) ↑ liver endothelial cell proliferation (by *ca* 45% at both sample times), ↑ Kupffer cell proliferation at 3 months (by 293%) but the control value was low at this sample time.
AA2935	♀ B6C3F1 mice treated with s47 s47 kg/day of pregabalin x 4 weeks and 5-6 mice/group were each assessed for liver cell proliferation as in study **AA2672**, terminal platelet activation (P-selectin) and terminal ADP-induced platelet aggregation also measured.	↑ platelet activation (HD, by 42%), ↓ end-point platelet aggregation with all doses (associated with the same maximal aggregation followed by disaggregation), no effect of treatment on liver cell (endothelial, Kupffer, hepatocyte) proliferation.
AA2990	♀ B6C3F1 normal (N) and platelet-depleted (PD) mice were treated with s47 mg/kg/day of pregabalin x 6 days and were killed on day 7 for assessment of liver cell proliferation as in study **AA2672**; platelet depletion was induced by IP neuraminidase s47 mouse in normal mouse serum on days -1, 2, 4, 6).	*Effect of pregabalin*: significant ↑ endothelial proliferation not demonstrated in N mice, but 31% ↑ in PD mice, ↑ hepatocyte proliferation in N (by 162%) and PD (by 72%) mice, Kupffer cell proliferation unaffected. *Platelet numbers*: ↓ by neuraminidase treatment on days 1 and 7 (by 83-87%).

In vivo (short term) - continued:

Study	Treatment* and measurements	Results
AA3012	♀ B6C3F1 mice were treated with s47 mg/kg/day of pregabalin and were killed on days 2, 3, 8, 15, 29; an additional group received IP phenylhydrazine s47 mg/kg on day 1 and 3) as a positive control for haemolysis-induced erythropoiesis; peripheral blood, serum, spleen, bone marrow examined for erythropoiesis indices and variables; pulmonary function also assessed.	pregabalin-related effects were generally seen at all sample times: *peripheral blood*: slightly ↑ erythroid values, ↑ platelet number (day 29, by 12%), ↑ platelet volume (by 7-15%), ↑ leukocytes (by 37-106%, reflecting ↑ neutrophils, lymphocytes), erythrocyte osmotic fragility unaffected by treatment. *bone marrow*: ↓ M:E (associated with ↓ M, ↑ E), ↑ % macrophages (days 15, 29; 4.5-7x higher), ↑ mouse incidence of erythrophagocytosis, ↓ PCE:NCE ratios (by 10-43%, acridine orange staining), no change in nucleated cell:E ratios. *spleen*: ↓ nucleated cell:E ratios (by 20-40%, reflecting ↑ % E, ↓ % nucleated cells). *serum*: minimal ↓ chloride (by 1.5-3%), ↑ bicarbonate (by 6-18%), no change in erythropoietin, bFGF, IL-3. *pulmonary function*: ↓ respiration rate (by 17-34%), ↓ minute volume, ↓ peak inspiratory and expiratory rates, tidal volume unaffected by treatment but noted to change in parallel with respiration rate during 60 min measurement period. *miscellaneous*: no change in liver 8-hydroxydeoxyguanosine content.
AA3053	♀ Wistar rats were treated with s47 mg/kg/day of pregabalin and were killed on days 2, 8, 15, 29; an additional group received IP phenylhydrazine s47 kg on day 1 and 3) as a positive control for haemolysis-induced erythropoiesis; peripheral blood, serum, spleen, bone marrow examined for erythropoiesis indices and variables.	pregabalin-related changes often occurred over the whole dosing period (terminal quantitative values are noted below): *peripheral blood*: slightly ↑ erythroid values apparent from first sample time, ↓ reticulocytes (by 42%), ↓ platelets and ↑ platelet volume (by 18-23%), ↑ platelet bFGF content (by 41%), erythrocyte osmotic fragility unaffected by treatment. *bone marrow*: no consistent changes in total nucleated cells or M:E ratios, ↓ megakaryocytes (by 66%, day 29), erythrophages not observed, PCE:NCE ratios unaffected by treatment. *spleen*: ↑ erythrocytes (by 46%), no change in PCE:NCE ratios, ↓ nucleated cells:erythrocyte ratio (by 38%). *serum*: ↓ glucose, cholesterol, chloride, sodium; erythropoietin unaffected by treatment. *miscellaneous*: no change in liver 8-hydroxydeoxyguanosine content.

* all by dietary administration; actual mean drug intakes were similar to nominal values
# PDGF = platelet-derived growth factor

In vivo (long-term):

Study	Treatment details*	Measurements and results
AA2787[a]	♀ B6C3F1 mice (56-61/group); s47 mg/kg/day x 12 or 24 months; 12 month treatment followed by 12 month recovery period.	terminal (week 104) values: *haematology*: no change in erythroid values, ↑ erythrocyte distribution width (HD, by 11%), no change in platelet numbers, ↑ platelet volume (MD and HD, by 13-38%), ↑ incidence of platelet aggregates (all doses), presence of giant platelets (HD). *bone marrow*: ↓ total nucleated cells (HD, by 17%), ↓ M:E ratios (MD and HD, by 27-43% reflecting ↓ absolute myeloid component), ↑ % macrophages (MD and HD, 3-5x control value), ↓ lymphocytes (HD, by 31%), ↑ megakaryocytes (HD, by 160%), ↑ incidence of erythrophagocytosis and presence of blood vessel segments (MD and HD). *serum growth factors*: ↑ PDGF (HD, by 47%), bFGF and VEGF unaffected by treatment. *respiratory function*[r]: ↓ BW-adjusted minute volume and tidal volume (by 41% and 36%). *haemangiosarcomas*[t]: 4/61 control, 4/61 LD, 7/61 MD, 15/57 HD (significant HD ↑); corresponding mortalities were 25%, 31%, 38%, 49%. *Recovery phase*: ↑ platelet volume (HD, by 6%), no group differences for bone marrow values, serum growth factors not measured, haemangiosarcoma incidences were 5/60 control, 6/61 LD, 5/60 MD, 10/56 HD (significant HD ↑, but HD incidence not significantly ↓ compared with 24 month treatment); corresponding mortalities were 37%, 31%, 38%, 32%.
AA2787[b]	♀ B6C3F1 mice; s47 mg/kg/day x 6 or 12 months; 6 month treatment followed by 9/10 week recovery period; 12 month treatment followed by 4 and 9/10 week recovery periods.	*end-of-treatment changes*: minimal ↑ erythroid values, minimal ↑ platelets, slightly ↑ platelet volume, ↑ leukocytes, ↑ platelet activation, ↓ terminal ADP-induced aggregation (associated with disaggregation), no change in plasma and platelet VEGF, PDGF and thrombopoietin, ↓ bone marrow M:E ratios (mainly reflecting ↑ erythroid component), ↑ megakaryocytes and % macrophages (12 months), ↑ liver endothelial cell proliferation at 12 months (MD by 260%, HD by 170%), ↑ Kupffer cell proliferation at 12 months (MD and HD, by *ca* 220%)[p]. *recovery periods*: limited data available, slightly ↑ platelet numbers at 4 and 10 weeks after 12 months treatment, platelet activation normalised, no bone marrow group differences at 10 weeks after 6 month treatment.
AA2892[c]	♀ CD-1 mice; s47 mg/kg/day x 3, 6, 12 months; 12 month treatment followed by recovery period.	*haematology*: ↑ HD platelet volume (6, 12 months), ↓ HD ADP-induced platelet aggregation (3, 12 months), ↑ HD platelet activation (6, 12 months), platelet morphology (size uniformity, hypogranularity, aggregates) unaffected by treatment. *bone marrow*: ↓ HD M:E ratios (3, 6, 12 months, reflecting ↑ erythroid component), ↑ HD % macrophages (3, 6, 12 months), megakaryocytes unaffected by treatment. *growth factors*: no effects of treatment on platelet/plasma PDGF, VEGF, thrombopoietin. *liver cell proliferation*[p]: no effects of treatment (3, 6, 12 months). *histology*[h]: hepatocytic necrosis at 3 months (1 LD, 4 MD, 3 HD) and 6 months (1 MD, 2 HD); bone marrow fatty change at 3 months (1 control, 1 LD, 2 MD, 5 HD) and 6 months (2 MD, 7 HD); NB ↑ BW (all doses) and food consumption (MD, HD) measured at 3 and 6 months.

In vivo (long-term) - continued:

Study	Treatment details*	Measurements and results
AA2934[d]	♂ and ♀ CD-1 mice; s47 mg/kg/day x 1, 3, 6 months.	*haematology*: ↑ platelet volume (all sample times, by 9-18%), ↑ ♀ platelets (6 months, by 14%), ↑ platelet activation (1 month, by 54-98%), ↑ abnormal ADP-induced platelet aggregation (secondary dissociation) although not clear-cut. *bone marrow*: ↓ M:E ratios (all sample times, by 35-45%), ↑ ♀ % macrophages (6 months, 14x higher), ↑ ♀ megakaryocytes (6 months, by 200%), ↓ lymphocytes (3 and 6 months, by 20-47%). *growth factors*: no consistent effects of treatment on platelet/plasma thrombopoietin, PDGF, VEGF. *liver cell proliferation*[p]: no effects of treatment on hepatocytes, endothelial and Kupffer cells. *histology*: bone marrow ↑ megakaryocytes (all sample times) and macrophages (3 and 6 months); spleen ↑ extramedullary haematopoiesis and presence of megakaryocytes (both at 3 and 6 months); no drug-related liver findings but lung foamy macrophages (3 and 6 months); ↑ immunoreactive VEGF in spleen red pulp and ♂ bone marrow (assessed only at 6 months).
AA2796[c]	♀ Wistar rats; s47 mg/kg/day x 1, 3, 6, 12, 18 months.	*haematology*: ↑ erythroid values (18 months, by 11-17%), ↓ % reticulocytes (all sample times, by 19-35%), ↑ platelet volume (all sample times, by 14-35%), platelet numbers/activation/aggregation unaffected by treatment. *bone marrow*: ↓ total nucleated cells at first sample time of 6 months (by 40%, associated with ↓ myeloid and erythroid cells, lymphocytes, megakaryocytes), no effect of treatment at 18 months. *growth factors*: platelet/plasma thrombopoietin, PDGF, VEGF unaffected by treatment, ↑ platelet rich plasma bFGF (all sample times, 2.9-7.1x control values). *liver cell proliferation*[p]: no effects of treatment on hepatocytes, endothelial and Kupffer cells. *histology*: ↑ bone marrow fatty infiltrate, ↑ lung foamy macrophages, no drug-related liver or spleen findings. *miscellaneous*: liver 8-hydroxydeoxyguanosine content unaffected by treatment (measured at 12 months).

* all by dietary administration; achieved mean drug intakes were close to nominal values

[a] interim report (**745-03832**), but containing all data

[b] interim report (**745-03657**) of different arm of study **AA2787**, with incomplete data

[c] interim report; data for a recovery phase not included

[d] interim report (**745-03766**); 1 and 3 month data also included in interim report **745-03658** but discrepancies for liver cell proliferation values between reports

[e] interim (**745-03463**) and final (**745-03763**) reports; discrepancies for liver cell proliferation values between reports

[p] liver cell proliferation assessed as in study **AA2672**

[t] liver, spleen, heart, skin, bone marrow examined

[r] 10 control and HD assessed in weeks 95/96 (24 month treated)

[h] liver, lung, spleen, bone marrow examined (10/group/sample time); findings were minimal-mild

11. REPRODUCTIVE AND DEVELOPMENTAL TOXICITY

Study type and details	Species/ strain/ group size	Doses (mg/kg/day)* and treatment duration	Findings
Fertility (♂) and early embryonic development. SN: **1935**. Date: June-Dec. 1994. Laboratory: Parke-Davis, Ann Arbor, MI, USA. GLP: Yes.	25 ♂ Wistar rats.	s47 [redacted] PO from 11 weeks prior to mating (with untreated ♀) and during a 19 day mating period to week 15 when 10 ♂/group were killed; pregnant ♀ killed on GD 13-15; 15 ♂/group kept for a 9 week recovery period after cessation of dosing during which they were mated weekly with 2 untreated ♀ and were killed in recovery week 10.	*Toxicity*: ↓ premating BWG (MD by 26%, HD by 51%; associated ↓ food consumption), hypoactivity (MD, HD). *Mating and fertility (week 11)*: mating incidence unaffected by treatment but ↑ MD and HD time to mating (3.6 and 5.3 days vs 2.6 days for controls), HD infertility (0/25 pregnancies), ↓ MD fertility (15/24 pregnancies vs 23/25 LD, 21/25 control). *Reproductive organ toxicity (week 15)*: ↓ MD and HD absolute (but not BW-relative) epididymidal weights (by 15-24%), ↓ MD and HD epididymidal (but not testicular) sperm counts (by 48-57%), ↓ vas deferens sperm motility with all doses (by 6%, 43%, 95%), ↓ MD and HD normal epididymidal sperm morphology, sporadic MD and HD histological findings (epididymidal luminal cell debris). *Litter values*: ↑ MD pre-implantation loss (36% vs 10% for controls) and ↓ number of live embryos. *Mating and fertility (recovery phase)*: mating unaffected by prior treatment, impaired fertility observed in week 1 (14 control, 15 LD, 11 MD, 1 HD ♂ produced at least one pregnancy; total numbers of pregnant ♀ were 22 control, 24 LD, 13 MD, 1 HD), tendency for slightly lower fertility in week 2 (total numbers of pregnant ♀ were 25 control, 30 LD, 22 MD, 20 HD) but not in subsequent weeks. *Reproductive organ toxicity (week 25)*: sperm number, motility, morphology had normalised. *Litter values (recovery phase)*: ↑ pre-implantation loss (MD and HD in weeks 1 and 2, HD in weeks 3 and 4), ↓ number live embryos (MD and HD in week 1, HD in weeks 2 and 3). *NOEL*: s47 [redacted] mg/kg/day (fertility).
Fertility (♂) and early embryonic development. SN: **2211**. Date: June-Sept. 1997. Laboratory: Parke-Davis, Ann Arbor, MI, USA. GLP: Yes (not signed by study director).	25 ♂ Wistar rats[&].	s47 [redacted] PO from 11 weeks prior to mating (with untreated ♀) and during the mating period; ♂ killed on day 109; pregnant ♀ killed on GD 13-15.	*Toxicity*: ↓ premating BWG over days 0-7 (all doses, by 12%, 23%, 33%; also ↓ MD, HD food consumption) and over days 0-77 (HD, by 7%), red fur staining (MD, HD). *Mating and fertility*: unaffected by treatment. *Reproductive organ toxicity*: reproductive organ BW-relative weights, sperm (number, motility, morphology), testes/epididymides histological findings unaffected by treatment. *Litter values*: corpora lutea, implantations, live embryos, resorptions, pre- and post-implantation losses unaffected by treatment. *NOEL*: s47 [redacted] mg/kg/day (fertility).

Continued:

Study type and details	Species/ strain/ group size	Doses (mg/kg/day)* and treatment duration	Findings
Fertility and early embryonic development. SN: **1899**. Date: Dec. 1993-Feb. 1994. Laboratory: Parke-Davis, Ann Arbor, MI, USA. GLP: Yes.	25 ♀ Wistar rats[&].	0, 500, 1250, 2500, PO from 15 days prior to mating (with untreated ♂) to GD 7; dams were killed on GD 13-15.	*Toxicity*: 1 HD premature death of unknown cause, clinical signs (transient hypoactivity, HD; urine staining, all doses), ↓ premating BWG (by 24-56%, non dose-related and non significant), GD 0-8 BWG unaffected by treatment, ↓ GD 8-13 BWG (MD by 69%, HD by 57%). *Oestrus cycling*: dose-related ↓ in number of cycles completed/15 days (2.64-2.04 vs 2.87 for controls), ↑ number with prolonged dioestrus, oestrus, proestrus. *Mating and fertility (pregnancies/mating)*: unaffected by treatment, except for ↑ number of days to mating with all doses (*ca* 3 days vs 1.9 days for controls). *Litter values*[$]: corpora lutea, implantations, number of live embryos unaffected by treatment, ↑ HD resorptions and post-implantation loss (19.8% vs 4.3% for controls), ↑ MD and HD pre-implantation loss (*ca* 10% vs 1.4% for controls). *NOEL*: s47 [redacted] mg/kg/day (fertility), s47 [redacted] mg/kg/day (embryofetal toxicity).
Fertility and early embryonic development. SN: **2586**. Date: Nov. 1999-Feb. 2000. Laboratory: Pfizer, Ann Arbor, MI, USA. GLP: Yes.	16/sex 7 day old Wistar rats.	s47 [redacted] PO x 7 weeks (premating period) and during the 19 day mating period; ♀ treatment continued to GD 7 and dams killed on GD 13-15; treated ♂ mated with untreated ♀ and *vice versa*.	*Toxicity*: no drug-related deaths, clinical signs (bruxism, hyperactivity, urine staining), times to vaginal opening and preputial separation unaffected by treatment, ↓ BWG over premating period for ♂ (MD by 12%, HD by 15%) and ♀ (MD and HD, by 6%, tendency[t]), ↓ treated ♀ BWG over GD 8-13 (MD by 21%, HD by 24%). *Mating and fertility*: unaffected by ♂ and ♀ treatment, NB ♀ fertility index was low in all treated groups - including controls (60-67%, 8-10 dams/group available for assessment). *Reproductive organ toxicity (♂)*: no effects of treatment on BW-relative testes, epididymides, accessory organ weights or sperm counts, minimal HD ↓ in sperm motility and normal morphology (by 3-5%). *Oestrus cycling*: prolonged dioestrus in 5 control, 6 LD, 9 MD, 10 HD ♀ (assessed over treatment days 35-49). *Litter values (untreated ♀)*: tendency[t] for ↓ number of live embryos and ↑ pre-implantation loss (HD). *Litter values (treated ♀)*: no clear effects of treatment, but low numbers of dams and high variability precluded proper assessment. *NOEL*: s47 [redacted] mg/kg/day (♂ and ♀ fertility).

Continued:

Study type and details	Species/ strain/ group size	Doses (mg/kg/day)* and treatment duration	Findings
Fertility (♀) and early embryonic development**. SN: **AA2772**. Date: May-Aug. 2001. Laboratory: Pfizer, Ann Arbor, MI, USA. GLP: Yes.	20 ♀ 7 day old Wistar rats.	s47 [redacted], PO x 7 weeks (premating period) and during the mating period to GD 7; dams killed on GD 13-15; treated ♀ mated with untreated ♂.	*Toxicity*: no drug-related deaths, hyperactivity with all doses, ↓ BWG during premating period (MD and HD, by 10%), time to vaginal opening unaffected by treatment. *Oestrus cycling*: prolonged dioestrus seen in 2 control, 2 LD, 5 MD, 7 HD rats (treatment days 35-49), number of affected controls ↑ to 6 for this period combined with the mating period. *Mating and fertility*: no effect of treatment on mating, tendency[t] for slightly ↓ MD and HD fertility index (75% and 71% vs 89% for controls, the HD value excludes 1 mated rat whose pregnancy status was not recorded in error). *Litter values*: no effects of treatment on numbers of corpora lutea, implantations, live embryos, pre- and post-implantation losses. *NOEL* s47 [redacted]ng/kg/day (fertility and litter values), although reduced fertility at higher doses may have been incidental.
Embryofetal development. SN: **1898**. Date: Jan.-Feb. 1994. Laboratory: Parke-Davis, Ann Arbor, MI, USA. GLP: Yes.	25 ♀ Crl:CD-1 (ICR)BR VAF/plus mice[&].	s47 [redacted], PO on GD 6-15; dams killed on GD 18.	*Maternal toxicity*: none. *Litter values*: no effects of treatment on post-implantation loss, number of live fetuses, fetal weight. *Fetal examinations*[#]: no effects of treatment. *NOEL*: s47 [redacted]mg/kg/day.
Embryofetal development. SN: **1893**. Date: Nov.-Dec. 1993. Laboratory: Parke-Davis, Ann Arbor, MI, USA. GLP: Yes.	20 ♀ Wistar rats[&].	0, 500, 1250, 2500, PO on GD 6-17; dams killed on GD 21.	*Maternal toxicity*: initial (GD 6-9) BW loss with all doses (by 1-2.5% vs a 3.9% control gain), ↓ BWG over GD 6-18 (by 12-25%, significant for HD), ↓ HD food consumption, clinical signs with all doses (hypoactivity, tail chewing, urine staining). *Litter values*: post-implantation loss and number of live fetuses unaffected by treatment, ↓ HD fetal weight (by 9%). *Fetal examinations*[#]: no treatment-related external or visceral findings, ↑ skeletal malformations[@]: jugal bone fused to maxilla (MD, HD) and nasal bones fused (HD), ↑ skeletal variations with all doses (middle phalanges ossification, extra well formed lumbar ribs, rudimentary cervical ribs, unossified ventral tubercle of atlas), ↓ number of ossification sites on cervical centra (HD). *NOEL*: s47 [redacted]mg/kg/day (maternal toxicity, fetal development) s47 [redacted]mg/kg/day (teratogenicity).

Continued:

Study type and details	Species/ strain/ group size	Doses (mg/kg/day)* and treatment duration	Findings
Embryofetal development. SN: **1907**. Date: Mar.-April 1994. Laboratory: Parke-Davis, Ann Arbor, MI, USA. GLP: Yes.	20 ♀ NZW rabbits[&].	0, 250, 500, 1250, PO on GD 6-20; dams killed on GD 30.	*Maternal toxicity*: 1 HD killed moribund with total resorption, 1 HD abortion, clinical signs with all doses (hypoactivity, ataxia, cool), ↑ BWG over GD 6-21 with all doses (by 32-44%). *Litter values*: no effects of treatment on post-implantation loss or number of live fetuses, HD fetal weight ↓ by 15%. *Fetal examinations*[#]: ↑ HD visceral, skeletal variations (apparently incidental, see text below), retarded ossification (HD). *NOEL* s47 ng/kg/day (developmental toxicity), s47 mg/kg/day (teratogenicity).
Pre- and post-natal development. SN: **1960** and **1962**. Date: Sept. 1994-Oct. 1995. Laboratory: Parke-Davis, Ann Arbor, MI, USA. GLP: Yes (not signed by study director).	25 pregnant Wistar rats[&].	s47 (MD), s47 (HD) s47 and s47 (LD1) s47 (LD2) PO, on GD 6-lactation day 20.	Marked toxicity occurred with the initial doses, and 2 lower doses with a concurrent control were consequently added to the study design. *Dams (gestation and parturition)*: 2 HD killed moribund on GD 21 and 22, dose-related clinical signs (ataxia, hypoactivity, bruxism, urinary staining), ↓ HD BWG over GD 6-21 (by 30%, with associated ↓ food consumption), slightly ↑ HD gestation length (by 0.7 days), dystocia (single MD, HD), killed on day 24 after failure to deliver (3 HD, 1 control), ↑ MD and HD stillborn/cannibalised pups (4.2 and 5.1/litter vs 0.3/litter for controls) and associated ↓ number of live pups. *Dams (lactation period)*: 16/24 MD and 16/16 HD killed because of total litter losses (by day 3 for HD), abnormal maternal care during early lactation (3 HD). *Offspring preweaning development*[d]: ↓ pup survival over lactation days 0-4 (LD (86%), MD (32%), HD (0%) vs 99% for controls) and 4-21 (MD), ↓ pup weights at birth (LD by 6%, MD by 14%, HD by 24%) and at weaning (LD2 by 8%, LD by 10%, MD by 37%), slightly delayed MD pinna detachment but no effect on times to eye opening or incisor eruption. *Offspring postweaning development*[e]: ↓ MD BW at week 13 (by 16% for ♂, by 5% for ♀), slightly delayed MD sexual maturation (testes descent, vaginal opening, preputial separation), no effects of dam treatment observed on activity or in rotarod and memory/learning tests, MD ♀ impairment of acoustic startle responses. *F1 reproductive performance (1/sex/litter)*[f]: mating and fertility unaffected by F0 dam treatment, ↓ MD maternal BWG during gestation (by 38% associated with ↓ food consumption), ↓ MD number of live fetuses on GD 21 associated with ↓ number of corpora lutea and ↑ pre-implantation loss, ↑ MD placental weight, no group differences for fetal weights or external findings. *NOEL*: s47 ng/kg/day (developmental toxicity).

* 0.5% methylcellulose vehicle
& plus additional rats for toxicokinetic measurements
# included visceral examinations of all fetuses by fresh dissection
@ litter incidences: 2/19 control, 1/18 LD, 4/18 MD, 10/18 HD (jugal bone/maxilla fusion); 4/18 HD (nasal bone fusion); see comment below, and section 13.8
$ ↑ HD resorptions but no ↓ in live embryos due to (non significant) higher number of corpora lutea; pre-implantation loss < a mean historical control value of 12.7% (97 dams in 1 study)
d no HD pups available for testing
P F1 pups culled to 4/sex/litter on lactation day 4; post-weaning behavioural tests carried out on 1/sex/litter
r assessed at 13 weeks of age; there were only 6 MD ♀ for assessment (vs 22-25 for other groups), of which 5 were pregnant and 4 had timed pregnancies
t tendency = not statistically significant
** repeat study carried out because of inconclusive effects of treatment on treated ♀ in **2586**

Some findings in the rabbit embryofetal development study (**1907**) require comments. Litter incidences of some visceral variations (decreased gall bladder size, azygous lung lobe absent, left carotid arising from innominate) were increased in the HD group (3-4/15 affected). Incidences were within the historical control range for 6 company studies, and were probably incidental, except for the former. This occurred in 5/98 HD fetuses (5.1%) in 3/15 litters (20%), but not in the controls, and although the fetal incidence was lower than that in one historical control study (5.9%), the highest litter incidence was 8.3%. Published (1993) historical controls values, collated by the Middle Atlantic Reproduction and Teratology Association (MARTA) were also included, but reduced gallbladder size was not specifically noted. Litter incidences of bent skull bone (variation) were variable (1/15 control, 5/16 LD, 2/16 MD, 6/15 HD) and did not appear to be treatment-related. Fused sternebrae were seen only in HD fetuses (2/98 in 2/15 litters), while this was noted in 1/68 fetuses in 1/12 litters in one company control study, but incidences were less than the maximum values in the MARTA historical control studies (respectively 3.4% and 15%).

Subsequent investigations indicated that malformations observed in the rat embryofetal development study (**1893**) were normal fetal developmental changes (see section 13.8).

Toxicokinetics

Plasma 4 h concentration (µg/mL) or $AUC_{0\text{-}24\,h}$ (µg.h/mL):

Study	Sampling day/week	No./dose/ sample time	Sampling times	**Dose** (mg/kg/day)
1935	week 15	5 ♂	4 h	s47 [redacted]
2211	day 109	5 ♂	0, 2, 4, 7, 12 h	
1899	day 13	5 ♀	4 h	
2586	day 28	6 ♂/6 ♀	4 h	
AA2712[$]	day 21	4 ♂/4 ♀	0, 1, 2, 4, 7, 12 h[@]	

Plasma 4 h concentration (µg/mL) or $AUC_{0\text{-}24\ h}$ (µg.h/mL) - continued:

Study	Sampling day/week	No./dose/ sample time	Sampling times	**Dose** (mg/kg/day)
AA2715#	day 22	3 ♂/3 ♀	0, 1, 2, 4, 7, 12 h	s47 [redacted]
1898**	GD 11	5 ♀	0, 1, 4, 7, 12 h	
1893	GD 15	5 ♀	0, 1, 4, 7, 12 h	
1907	GD 14	4 ♀	0, 1, 2, 4, 6, 12 h	
1960 and **1962**	lactation days 12-14	6 ♀	0, 1, 4, 7, 12 h 4 h (milk)*	

& respective 1250 and 2500 mg/kg/day values for 1-2 dams were 6170 and 8930 µg.h/mL
* 4 h milk/plasma concentration ratios were 1.2-1.6 $ 3 week toxicokinetic study using 7-day old Wistar rats
# 3 week toxicokinetic study using adult Wistar rats ** respective C_{max} values were 291, 640, 1310 µg/mL
@ t_{max} = 1 h

12. LOCAL TOLERANCE

Study type and details	Treatment details	Examinations and findings
IV irritation. SN: **2309** Date: May 1998 Laboratory: Parke-Davis, Ann Arbor, MI, USA. GLP: Yes (not signed by study director)	♀ NZW rabbits (5/group) received 1 mL IV injections of 20 mg/mL pregabalin or saline vehicle, daily x 5 days; injection sites examined grossly and histologically.	no signs of local irritation observed; no clinical reactions or gross findings at necropsy.
Blood compatibility *in vitro*. SN: **2317** Date: May 1998 Laboratory: Parke-Davis, Ann Arbor, MI, USA. GLP: Yes (not signed by study director)	0.2-10 mg/mL of pregabalin tested for precipitation or coagulation of plasma proteins, haemolysis and effects on erythrocyte hypo-osmotic fragility (human heparinised blood).	no effects of treatment.
Contact sensitisation (local lymph node assay). SN: **2650** Date: April-May 2000 Laboratory: Parke-Davis, Ann Arbor, MI, USA. GLP: No.	♂ Wistar rats were topically treated with s47 [redacted] pregabalin in s47 [redacted] on days 1-4 and were killed on day 7; draining lymph node cell number and type (T-, B-, % blasts), ^{3}H-thymidine uptake, BrdU-uptake (% of cells in S-phase) measured; positive (1% oxazolone) and negative (25% methylsalicylate) controls included.	no effects of pregabalin, but marked responses with oxazolone; s47 [redacted]

13. OTHER TOXICITY STUDIES

13.1 Antigenicity

No studies.

13.2 Immunotoxicity

No studies.

13.3 Dependence

Report	Experimental model and details	Results
740-03524	i) 4 ♂ rhesus monkeys that discriminated between midazolam and vehicle injections used; correct lever pushing measured. ii) 3 diazepam-dependent rhesus monkeys that could discriminate between vehicle and flumazenil (benzodiazepine antagonist, a range of doses tested) used; correct lever pushing measured.	i) pregabalin s47 [redacted] prior to testing) did not elicit midazolam-lever pushing. ii) prior (4 h) PO pregabalin treatment gave inconsistent results.
770-00314	rats trained for morphine-associated conditioned place preference (MA-CPP) in enclosure box were used to test effects of pregabalin.	pregabalin s47 [redacted] inactive in eliciting MA-CPP; prior PO treatment with 10 mg/kg (but not 1, 3, 30 mg/kg) pregabalin ↓ MA-CPP response to morphine; prior SC pregabalin (10 mg/kg) attenuated morphine elicited ↑ in nucleus accumbens extracellular dopamine concentrations (microdialysis probe).
740-03441	anti-abuse potential assessed by antagonism of cocaine- and amphetamine-stimulated locomotor activity in rats; PO treatment s47 [redacted] mg/kg) at 1 h prior to testing s47 [redacted] prior to stimulant).	↑ activity elicited by both stimulants abolished with HD; also antagonised by GABA (activity ↓ to < baseline value at s47 [redacted]
745-03278*	elicited clinical signs and self IV-administration (sequential sessions with saline, pentobarbitone, saline, pregabalin, saline) assessed in ♀ rhesus monkeys.	high self-administration of pentobarbitone (1 mg/infusion) but not of pregabalin s47 [redacted] mg/kg/infusion).
740-03525	re-enforcing effect of pregabalin s47 [redacted] mg/kg/infusion) substitution in rhesus monkeys self IV administering methohexitone s47 [redacted] mg/kg/infusion).	mean number of injections/session: s47 [redacted] (methohexitone) s47 [redacted] saline), s47 [redacted] (pregabalin, not dose-related).
740-03540	potential withdrawal symptoms assessed in ♂ rats over 4 days following continuous 12 day IP infusion with saline, pregabalin (initial/final: s47 [redacted] or pentobarbitone (initial/final: s47 [redacted] mg/kg/day).	BWG over 0-96 h: 2 % (saline): -8.9% (pentobarbitone), -5.9% (LD), -6.6% (MD), -5.3% (HD). clinical withdrawal scores (arbitrary units): 1.1 (saline), 14.1 (pentobarbitone), 3.6 (LD), 6.0 (MD), 4.2 (HD). conclusion: some withdrawal apparent, but much less pronounced than with pentobarbitone.

* GLP-compliant

13.4 Metabolites

No studies.

13.5 Studies on impurities and degradation products

13.5.1 Rats

Study details	Strain and group size	Dose (mg/kg/day) and duration	Findings
SN: **1654**. Date: Mar.-July 1999. Laboratory: Parke-Davis, Mississauga, Ontario, Canada. GLP: Yes.	Wistar, 10/sex*.	s47 [redacted]	convulsions in 1/10 HD ♀, tendency for ↓ BWG (MD, HD; by 4-14%), no effects of treatment on ophthalmic observations, haematology, serum chemistry, urinalysis, BW-relative organ weight values, gross and histological findings, bone marrow cell counts and numbers of micronucleated PCE# (5/sex/group assessed for both variables).
SN: **1616**. Date: Oct.-Nov. 1997. Laboratory: Parke-Davis, Mississauga, Ontario, Canada. GLP: Yes.	Wistar, 10/sex*.	s47 [redacted] weeks	there were no effects of treatment on mortality, BWG, ophthalmic observations, haematology, serum chemistry, urinalysis, BW-relative organ weight values, gross and histological findings, bone marrow cell counts and numbers of micronucleated PCE# (5/sex/group assessed for both variables).

* plus additional 6/sex for toxicokinetic measurements
& 0.5% methylcellulose vehicle
# validated with a positive control

Toxicokinetics

Plasma AUC (µg.h/mL) - sex-combined&:

Study	Sampling week	No./sex/dose /sample time	Sample times	Dose (mg/kg/day)
1654 (separate report 764-03384)#	12	3	predose, 1, 4, 7, 12, 24 h (0-24 h)	s47 [redacted]
1616	4	3	predose, 1, 4, 7, 12, 24 h (0-last)	s47 [redacted]

s47 [redacted]

\# no conversion to S-enantiomer (pregabalin) observed at t_{max} (1 h)
* unreliable due to excessive extrapolation

13.5.2 Genotoxicity

Test type and details	Test system	Test conditions*	Results	Validity
Bacterial reverse gene mutation. SN: **2472**. Date: May-June 1999 Laboratory: s47 [redacted] GLP: Yes.	*S. typhimurium*, strains TA 100, 98, 1535, 1537; *E. coli* WP2*uvr*A.	s47 [redacted] µg/plate of the R-enantiomer tested, with and without S9 activation, in 2 separate assays.	**Negative** no indications of bacterial toxicity.	↑ revertant colonies with positive controls.
Bacterial reverse gene mutation. SN: **2295**. Date: April-May 1998 Laboratory: Parke-Davis, Ann Arbor, MI, USA. GLP: Yes.	*S. typhimurium*, strains TA 100, 98, 1535, 1537; *E. coli* WP2*uvr*A.	s47 [redacted] µg/plate of the lactam degradant (PD 147804) tested, with and without S9 activation, in 2 separate assays.	**Negative** bacterial toxicity at s47 [redacted] µg/plate -S9.	↑ revertant colonies with positive controls.

* hepatic S9 preparations from Aroclor 1254-treated rats

13.6 Developmental neurotoxicity

Study details	Strain and group size	Dose (mg/kg/day)*	Measurements and findings
SN: **AA2589** Date: Dec. 1999-Mar. 2000. Laboratory: Pfizer, Ann Arbor, MI, USA. GLP: Yes.	7 day old Wistar rats, 15/sex.	s47 [redacted] PO x 7 weeks (49 days); rats killed on days 80-83.	No premature deaths, clinical signs (hyperactivity, hyper-reactivity, bruxism; all doses), ↓ BWG over treatment period (♂ all doses, ♀ HD; by 9-15%), no effects of treatment on times to incisor eruption and eye opening or visual placing (day 14) and rotarod (day 21) performance, acoustic startle response changes (day 35) including ↓ maximum responses in ♂ (by a mean 33-55% with all doses for the first 3 of multiple trials), sporadic minor differences in activity values (day 42) including ↑ ♂ initial stereotypy time with all doses, no effects of treatment on ♀ Morris water maze behaviour (days 28-30, 49-51, 77-79)[&] while HD ♂ behaviour was impaired on days 28 and 29 only, no treatment related gross findings at necropsy.
SN: **AA2861**[#] Date: Feb.-April 2002 Laboratory: Pfizer, Ann Arbor, MI, USA. GLP: Yes.	7 day old Wistar rats, 16/sex.	s47 [redacted] (MD1), s47 [redacted] (MD2) s47 [redacted] PO x 7 weeks (49 days); rats killed in week 10.	No treatment-related premature deaths, clinical signs (hyperactivity, hypoactivity, bruxism; all doses), ↓ BWG over treatment period (MD2 and HD, by 7-17%), no functional observation battery findings consistent between testing times during (2, 4, 6) or seen after (week 8) the treatment period (↑ alertness noted in weeks 4 (♂), 2 (♀)), maximum acoustic startle responses (week 8) impaired in MD2 and HD ♂ (by a mean 38-61% for the first 3 of 50 trials), activity (week 9) unaffected by treatment, impaired Morris water maze behaviour (weeks 9-10) in HD ♀, no treatment-related gross findings or brain histological changes (control and HD rats examined).

* 0.5% methylcellulose vehicle [&] median times to find a platform measured; first day was for training, second and third days were for testing (learning and memory assessed)
[#] repeat study with behavioural measurements primarily after cessation of treatment

13.7 Haematological, vascular and dermal toxicity

Study details	Strain and group size	Treatment and measurement details	Results
SN: **1597** Date: Dec. 1996-July 1997. Laboratory: Parke-Davis, Mississauga, Ontario, Canada. GLP: Yes.	♂ Wistar rats, 20.	s47 mg/kg/day PO*; phase 1: 5/group killed after 1, 2, 5, 14 days for haematological/platelet aggregation/femoral bone marrow measurements and limited histological examinations; phase 2: rats treated IV with sulpho-NHS-biotin on day 14 and 4/group killed after 1, 2, 3, 4, 5 days for the measurement of labelled platelets; haematological values also determined.	slight and sporadic ↑ erythroid values, ↓ platelet count (phase 1 rats only), ↑ platelet volume (all with HD); tendency for ↑ tail tip bleeding time (LD, MD); ↓ BW-relative spleen weights (by 13%, no associated histological findings); ↑ day 12 serum erythropoietin in 2/5 LD and 1/5 HD rats. no effect of treatment on platelet lifespan (*ca* 4.5 days), PT and APTT, platelet aggregation/ATP release reaction (terminal collagen-, ADP-, thrombin-induced), bone marrow values (including M:E ratios, number of megakaryocytes), bone marrow (sternum, femora) and spleen histology, platelet and megakaryocyte ultrastructure (no data). *toxicokinetics*: 4 h (phase 1, day 14) plasma drug concentrations were s47 LD) and s47 (HD) µg/mL.
SN: **2615** Date: Feb.-Mar. 2000. Laboratory: Parke-Davis, Ann Arbor, MI, USA. GLP: No.	Wistar rats, 10/sex.	rats treated with s47 s47 mg/kg/day x 2 weeks (diet); haematological, bone marrow, platelet aggregation, haemostasis measurements carried out.	*haematology*: ↑ ♂ erythroid values (all doses), slightly ↓ platelets (by 4-12%, all doses), ↑ ♂ platelet volume, slightly ↑ % of platelets with abnormal morphology[&] (all doses), ↑ ♂ leukocytes and absolute lymphocytes (all doses, resp. by 18-64% and 25-80%, not-dose related). *bone marrow (flow cytometry)*[#]: ↑ ♀ M:E ratios (all doses), ↓ ♀ absolute myeloid cells (MD and HD, by *ca* 24%), ↓ ♀ absolute erythroid cells (all doses, by 35-46%), ↓ ♀ absolute megakaryocytes (all doses, by 64-76%, tendency for smaller ↓ in ♂)[$]. *platelet function*: no effect of treatment on platelet activation (P-selectin), % reticulated platelets, ADP-induced aggregation and release reaction; ↓ collagen-induced aggregation (HD, by *ca* 30%). *haemostasis*: no effect of treatment on ear template bleeding times (measured pretest and day 14), % clot retraction.
SN: **2686** Date: July 2000. Laboratory: Parke-Davis, Ann Arbor, MI, USA. GLP: No.	♂ Wistar rats, 8 in total.	extravasation of IV-administered [^{125}I]albumin measured at 1 h after 100 µL intradermal injections of 0 (saline), s47 µg/mL pregabalin; histamine positive control also included.	no effect of pregabalin measured.

Continued:

Study details	Strain and group size	Treatment and measurement details	Results
SN: **SP1655** Date: April 1999-Feb. 2000. Laboratory: Parke-Davis, Mississauga, Ontario, Canada. GLP: No.	cyno. monkeys, 1-2/sex.	pregabalin treatment by continuous IV infusion (6 mg/kg/h); animals killed after 24, 48, 72 h (2/sex each) or 96 h (1/sex, also 1/sex saline controls) for assessment of factors involved in and characteristics of dermal toxicity.	oedema/swelling and dermatopathy (72 and 96 h); ↑ leukocytes and neutrophils (all sample times); no change in lymphocyte subsets (CD4, CD8, CD20) or 96 h bone marrow values; ↓ serum albumin; no changes in immunoglobulins, C-reactive protein, complement C3c, P-selectin, E-selectin, VCAM-1, cryoglobulin/anti-nuclear and anti-platelet antibodies (all absent); ↑ 96 h urinary protein. *skin histology*[@]: vasculopathy, oedema, ulcers; ultrastructural endothelial degeneration, thrombosis, perivascular oedema of time-dependent severity. *immunostaining of cutaneous blood vessels:* ↑ transmural staining for blood factor VIII, ↑ luminal staining for CD61 (platelets). *toxicokinetics*: mean plasma concentrations at 24-96 h were s47 µg/mL. *comment*: the small group size and inter-animal variation precluded proper comparisons.

* 0.5% methylcellulose vehicle

[&] presence of giant platelets, ↓ granularity and/or ↑ granule size

[#] individual values were within historical reference range

[$] megakaryocyte number (and size and morphology) in cytospin preparations said to be unaffected by treatment, but these data were not given and the basis for this difference was not clear

[@] lymph nodes, bone marrow, spleen, nasal turbinates also examined with no obvious effect of treatment

13.8 Special reproductive toxicity

Study type and details	Strain and group size	Treatment	Results
Effects on sperm production and quality. SN: **2159** Date: Nov. 1996-June 1997. Laboratory: Parke-Davis, Ann Arbor, MI, USA. GLP: Yes (not signed by study director).	♂ Wistar rats, 56 or 60.	phase s47 mg/kg/day PO*; 6-7/group killed at weekly intervals to 6 weeks for sperm assessment (3/group also for testes/epididymides electron microscopy examination). phase 2 (reversibility): 0 or s47 ng/kg/day PO* x 3 weeks; 7/group killed at end-of-treatment and weekly x 6 weeks.	*phase 1*: ↓ BWG (by 61% over weeks 1-6), no effect on BW-relative testes and epididymides weights, ↓ cauda sperm count over weeks 2-6 (by 30-58%, sperm/g), minimal ↓ in caput sperm counts (by 17% at 6 weeks), ↓ sperm motility in vas deferens (by 57% at 2 weeks to virtually all non-motile in weeks 5-6) and proximal cauda (by 75-95% over weeks 3-6), ↓ % normal sperm in cauda over weeks 2-6 and caput over weeks 4-6 (resp. associated with detached heads and abnormal heads/flagella). *phase 2*: changes were fully reversible by recovery week 6, sperm motility and morphology started to normalise before sperm counts. *toxicokinetics*: the mean 4 h plasma drug concentration in treatment week 5 (phase 1, 5 rats) was s47 µg/mL. *ultrastructure*[&]: no treatment-related testicular findings; abnormalities of epididymides and sperm, including luminal debris, sperm multiple tails and mitochondrial disorganisation.
Effects on sperm motility *in vitro*. SN: **2022** Date: June-Oct. 1995. Laboratory: Parke-Davis, Ann Arbor, MI, USA. GLP: Yes.	♂ Wistar rats, 6 in total.	4 vas deferens segments from each rat incubated with 0, s47 µg/mL of pregabalin or a positive control s47 µg/mL) x 7 h at 37 °C; sperm motility and morphology	pregabalin was without effect, marked ↓ motility with the positive control.
♂-Mediated embryofetal toxicity. SN: **AA2603** Date: Jan.-May 2000. Laboratory: Pfizer, Ann Arbor, MI, USA. GLP: Yes.	♂ Wistar rats, 25.	treatment with s47 , s47 mg/kg/day PO* from 11 weeks prior to and during a mating period; untreated females killed on GD 21.	*toxicity*: clinical signs (bruxism, hyperactivity; all doses), ↓ BWG over premating period (MD by 15%, HD by 8%). *mating and fertility*: unaffected by treatment. *litter values*: no effects of treatment on implantations, number of live fetuses, pre- and post-implantation loss; MD and HD ♂ fetal weights were slightly ↓ (by 4.5%) but were within the historical control range and ♀ were unaffected. *fetal findings*: fetal/litter incidences of variations unaffected by treatment, fetal/litter incidences of total malformations tended to ↑ with dose (3/2 control, 4/3 LD, 5/5 MD, 11/7 HD) but no particular external, visceral or skeletal change was ↑. *NOEL (embryofetal toxicity)*: 500 mg/kg/day. *toxicokinetics*: $AUC_{0\text{-}24\,h}$ values in treatment week 14 were s47 µg.h/mL.

Continued:

Study type and details	Strain and group size	Treatment	Results
Fetal skull development. SN: **AA2621** Date: Mar.-April 2000. Laboratory: Pfizer, Ann Arbor, MI, USA. GLP: Yes	pregnant Wistar rats, 25.	treatment with 0 (vehicle), s47 s47 mg/kg/day PO* on GD 6-17; dams killed on GD21; an untreated control group also included; fetal skulls examined by skeletal staining (1/2) and light microscopy of jugal bones (1/2, untreated, vehicle control and HD).	*maternal toxicity*: ↓ BWG over GD 6-18 at ≥100 mg/kg/day (respectively by 7%, 17%, 8%, 10%, 30%), BW loss over GD 6-9 with s47 ng/kg/day (by 1.4-2.7% vs a 4% gain for vehicle controls). *litter values*: ↑ HD resorptions (2.6/dam vs 1.0/dam for vehicle controls) and slightly ↓ number of live fetuses. *fetal skull (stained)*[#]: jugal bone fused to maxilla (litter incidence): 3/24 (untreated), 2/23 (vehicle), 4/23, 2/25, 4/23, 4/23, 12/24, 12/23; nasal bones fused: ↑ with HD (7/23 litters vs 0/23 vehicle controls and 1-2 litters at some intermediate doses). *fetal skull (histology)*: unremarkable jugal sutures (zygomatic arch).
Fetal skull development. SN: **AA2646** Date: Mar.-Sept. 2000. Laboratory: Pfizer, Ann Arbor, MI, USA. GLP: Yes	pregnant Wistar rats, 120.	treatment with s47 mg/kg/day PO* on GD 6-17 (GD 0 = post-mating (PM) day 0); dams and offspring killed daily over PM days 19-26 (10 or 20/group); fetal and pup skulls stained and closure of jugal bone and nasal sutures recorded.	*maternal and pup toxicity*: ↓ maternal BWG over treatment period; one dam killed on PM day 23 with dystocia, ↑ dead/cannabilised/missing pups on days 22-24 (3x, time-groups combined); 2/10, 4/10, 4/10 dams respectively scheduled to be killed on days 24, 25, 26 had no live offspring. *jugal suture*[$]: fetuses with closure ↑ from 0.85% on PM day 19 to 7.0% on PM day 26 (controls) or from 2.0% to 32% (pregabalin-treated); the highest control litter incidence was 6/10 (day 26). *nasal suture*[$]: 0-0.85% of control fetuses showed closure over PM days 19-24 with slight ↑ on days 25 and 26 (2%, 1.6%); values with pregabalin treatment ↑ from 2.2% on PM day 19 to *ca* 10% on PM days 21-25; the highest control litter incidence was 2/10 (day 26). *conclusion*: suture closures are variations not malformations, and are accelerated by pregabalin treatment.
Post-natal skull development. SN: **AA2604** Date: Feb.-April 2000. Laboratory: Pfizer, Ann Arbor, MI, USA. GLP: Yes	pregnant Wistar rats, 25.	treatment with s47 mg/kg/day PO* on GD 6-17 and dams allowed to litter; pups were killed at weaning and stained skulls examined; number of fetuses/litters examined: 284/22 (control), 214/19 (pregabalin-treated).	*maternal toxicity and pup development*: ↓ BWG over GD 6-18 (by 33%), whole litter loss (4/25 on lactation days 0-2 vs 0/24 controls), ↑ dead/cannibalised/missing pups (54 vs 10 controls), ↓ pup weights at birth (by 8%) and at weaning (by 6%). *pup jugal suture closed (fetal/litter incidence)* : 99.6%/100% (control), 94.8%/100% (pregabalin-treated). *pup nasal suture closed (fetal/litter incidence)*: 1.1%/13.6% (control), 4.7%/31.6% (pregabalin-treated). *other pup skull findings*: single case of misshapen skull (pregabalin-treated).

Continued:

Study type and details	Species, strain and group size	Treatment	Results
Developmental toxicity. SN: **2123** Date: June-July 1996. Laboratory: Parke-Davis, Ann Arbor, MI, USA. GLP: Yes (not signed by study director).	pregnant Wistar rats, 6.	treatment with s47 mg/kg/day PO* on: a) GD s47 b) GD s47 (P) c) parturition-LD 10 (LD 0= day of birth) d) s47 (also with vehicle).	i) for dams treated on s47 here were no surviving offspring by LD 2, ↑ number of stillborn pups (8x the control value), ↓ pup birthweight (by 26%), similar results for dams treated on s47. ii) for dams treated on s47 one had total resorptions and one had no surviving offspring by s47 pup survival at birth and over LD 4-10 unaffected by treatment, slightly ↓ pup weights at birth (*ca* 5%) and s47 by *ca* 3%). iii) one dam treated over s47 also had total litter loss on LD 4, pup survivals over s47 unaffected by treatment, ↓ pup weights at birth (by a 3%) and to a greater extent on s47 (by 32%). *conclusion*: adverse pup survivals mainly affected by treatment in late gestation.

* 0.5% methylcellulose vehicle
& separate study **AA2159**
# jugal bone fused to maxilla = closure of suturae zygomaticomaxillaris, nasal bones fused = closure of nasofrontalis
$ mean % of fetuses/litter affected, NB only 5-6 pregabalin-treated litters available for assessment on days 25 and 26

13.9 Retrospective histological examinations

Report No.	Examinations	Results
745-03280	kidney sections from ♂ rats treated with control diet or s47 /kg/day x 13-52 weeks, s47 kg/day x 13 weeks, s47 /kg/day x 4 weeks examined for hyaline droplets by fluorescence microscopy; samples from toxicity studies **1554** and **AA1994**.	slightly ↑ hyaline droplet severity with s47 mg/kg/day x 4 weeks.
745-03359	whole testes sections from rats treated with s47 mg/kg/day x 52 weeks (study **AA1994**) examined for stages of spermatogenesis.	spermatogenesis unaffected by treatment; slight non-dose related inhibition of spermiation in treated groups (1/15 LD, 4/14 MD, 2/14 HD) was not confirmed by a consultant reviewer.
745-03431	protocol-listed tissues from control and HD animals in the 13 week mouse (**1578**) and 65-69 week cynomolgus monkey (**AA1992**) toxicity studies examined by a consultant for evidence of vascular proliferative lesions.	no vascular proliferative lesions observed in either species.
745-03828	immunohistochemical staining for Flk-1* carried out on liver sections from surviving control and HD cynomolgus monkeys in the 65-69 week toxicity study (**AA1992**).	minimal-moderate sinusoidal endothelial cell staining unaffected by treatment.

Continued:

Report No.	Examinations	Results
745-03852	retinas from all cynomolgus monkeys in the 65-69 week toxicity study (**AA1992**) examined for abnormalities by an external consultant.	no effects of treatment observed.
745-03298	retinas from control and HD rats in the pivotal toxicity study (**AA1994**) and from all rats in a carcinogenicity study (**AA2235**) were examined by an external consultant.	bilateral retinal atrophy not seen in the pivotal study; ↑ incidences in the 104 week carcinogenicity study (65/sex/group): control LD MD HD ♂ s47 [redacted] ♀ except for 3 cases, atrophy was mild to moderate; treatment-related ↑ was considered to be a non-specific exacerbation of an age-related change.
745-02999	skin lesions from cynomolgus monkeys in a 2 week continuous IV toxicity study (**SP1644**) were examined by 2 expert external consultants.	the main conclusions were: i) primary change appeared to be endothelial cell damage and thrombosis in skin and mucous membrane superficial blood vessels. ii) most prominent findings were in capillaries, venules and arterioles of superficial dermis. iii) lesions were localised, with no evidence of disseminated intravascular coagulation in lungs and kidneys. iv) epidermal/dermal degeneration and necrosis were secondary to infarction resulting from vascular damage; oedema may have had a similar cause.

* Flk-1 = vascular endothelial growth factor receptor type 2, expressed on endothelial cells

13.10 Miscellaneous toxicity

13.10.1 Rabbits

Study	Strain and group size	Dose (mg/kg/day) and duration	Results
SN: **AA2644** Date: May-April 2000. Laboratory: s47 [redacted] GLP: Yes	5 ♂ NZW rabbits	s47 [redacted] weeks.	no premature deaths, ataxia (all doses), hypoactivity (MD, HD), ↑ BWG with all doses (by 60-87%, significant with MD and HD), sporadic ↑ food consumption, necropsy findings unaffected by treatment, clinical pathology measurements and histological examinations not carried out.

* 0.5% methylcellulose vehicle

13.10.2 Occupational safety

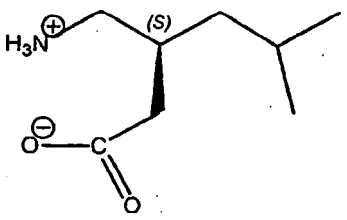
The following GLP-compliant studies are briefly summarised.

Report No.	Study type	Main results
	Racemate [redacted]	
901-00517	acute PO toxicity in rats	no mortalities at [redacted] mg/kg
901-00542	acute dermal toxicity in rabbits	no effects of dermal treatment with [redacted] mg/kg x 24 h
901-00520	dermal irritation in rabbits	negligible irritation with [redacted] g x 4 h
901-00508	ocular toxicity in rabbits	[redacted] was slightly irritating, with and without rinsing at 30 s.
901-00529	guinea pig skin sensitisation (modified Buehler method)	no sensitisation observed
901-00599	*S. typhimurium* reverse mutation assay	no activity seen with [redacted] µg/plate, with and without rat liver S9 activation
	Pregabalin mandelate [redacted]	
901-00717	acute PO toxicity in rats	no mortalities at [redacted] mg/kg; clinical signs included waddling gait, lethargy, unsteadiness
901-00718	acute dermal toxicity in rabbits	no effects of dermal treatment with [redacted] mg/kg x 24 h
901-00719	dermal irritation in rabbits	no irritation seen with [redacted] x 4 h
901-00720	ocular irritation in rabbits	[redacted] was moderately irritating, ↓ severity with rinsing at 30 s
901-00721	guinea pig skin sensitisation (modified Buehler method)	no sensitisation observed
901-00660	*S. typhimurium* reverse mutation assay	no activity seen with [redacted] µg/plate, with and without rat liver S9 activation

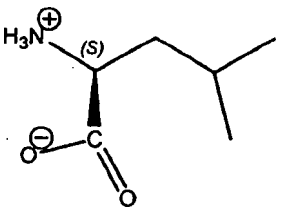
* synthetic intermediate

APPENDIX 1

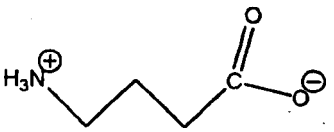
Pregabalin and related compound structures



Pregabalin



L-Leucine



GABA

* ^{14}C-labelling position

histological findings, but marked lesions (haemorrhagic mucosal necrosis, epithelial hyperplasia) occurred only in premature deaths in short term studies with high doses. Only lamina propria haemorrhages (minimal-moderate) were seen in the rat pivotal study (high-dose males affected), while no bladder lesions were apparent in the mouse or rat carcinogenicity studies. Increased uterine weights associated with gross and histological distension were seen only in the 2 rat carcinogenicity studies, with a clear effect at the high-dose (ER value of *ca* 24), while increased lung alveolar macrophages in rodents possibly reflected phospholipidosis. There were indications of cardiotoxicity in an initial 4 week study in cynomolgus monkeys, with findings of increased heart weights, heart enlargement/ventricular hypertrophy and histological degeneration/necrosis/fibrosis. This was thoroughly investigated in the subsequent pivotal study, which included additional ECG, echocardiography and blood pressure measurement, with no myocardial findings.

Tissue distribution studies showed high pancreatic concentrations of radioactivity after PO administration of [^{14}C]pregabalin to mice and rats, but not cynomolgus monkeys. However, this was not associated with specific toxicity in this organ, although there were some minor sporadic findings in rats (e.g. decreased acinar granules). [

]

Genotoxicity and carcinogenicity. No pregabalin genotoxicity was apparent in adequate assays, which included *ex vivo* unscheduled DNA synthesis and *in vivo* micronucleus formation in the 2 strains of mice used in the carcinogenicity studies. PO gavage doses were up to 2000 mg/kg, also used in the corresponding rat tests, which would have been expected to have achieved high plasma concentrations (>50x based on comparison of C_{max} or 4 h values in the reproductive toxicity studies with a human C_{max} value of 9.1 µg/mL). *In vitro* assay concentrations were to 5000 µg/plate (bacterial reverse mutation) or 1600 µg/mL (mammalian cell gene mutation, chromosomal aberrations), which is equivalent to *ca* 10 mM.

However, increases in haemangiosarcoma incidences were seen in B6C3F1 mice used in an original study, and to a lesser extent in CD-1 mice used in a confirmatory study conducted at a different laboratory, with both sexes being affected. Significant increases were apparent with doses expected to achieve ER values of *ca* 6-33 (B6C3F1, mid- and high-dose) and *ca* 28 (CD-1, high-dose), but a similar responses was not observed in 2 Wistar rat studies in which expected ER values were up to *ca* 25 in females. In a further study (AA2787), it was shown that increases in female B6C3F1 mice haemangiosarcoma incidences occurred after 1000 mg/kg/day treatment for 104 weeks or for 52 weeks followed by a 52 week recovery period. Although clear increases were demonstrated, this tumour was seen in control mice at incidences of 3-8.3% (B6C3F1, both sexes in the main study and females in 2 arms of study AA2787) and 3-9.2% (CD-1, male and female). There were no other treatment-related oncogenic findings.

The lack of demonstrated genotoxicity is suggestive of an epigenetic mechanism for this tumour, which arises from vascular endothelial cells and which was most prominent in the liver, spleen, bone marrow and uterus (CD-1 only). Endothelial cell and platelet functions are known to be intimately linked and as noted above, associated changes in the mouse carcinogenicity studies included increased platelet numbers and volume, and in B6C3F1 mice, increased bone marrow hypercellularity. Morphological abnormalities of platelets and the presence of schistocytes were also seen, with the latter probably reflecting changes in shear stress related to endothelial abnormalities.

Subsequently a number of *in vitro/ex vivo* and *in vivo* mechanistic studies were carried out in which there was little or no evidence for pregabalin acting as an endothelial cell mitogen, altering platelet function or stimulating megakaryocyte development *in vitro*. Different treatment

NONCLINICAL EVALUATION OF REGISTRATION APPLICATION

NEW CHEMICAL ENTITY

Sponsor:	**Pfizer Australia Pty Ltd**
Generic name:	**Pregabalin**
Trade name:	**LYRICA™**
Drug class:	**Analgesic, Anti-epileptic**
Dose form and strengths:	**25 mg, 50 mg, 75 mg, 100 mg, 150 mg, 200 mg, 225 mg, 300 mg capsules**
Submission No:	**2004-0021-1**
Toxicology file No:	**2004/009570**
Evaluator:	
Date:	**29 October 2004**

Note: This evaluation has been checked for confidential information and is cleared for release to the Sponsor only after this cover page and page 8 are replaced with the attached edited pages.

Document 2

pregabalin (Lyrica)

Australian Government
Department of Health and Ageing
Therapeutic Goods Administration

Nonclinical Evaluation Report

Pregabalin [LYRICA®]

Submission No: 2006-0097-1

Sponsor: Pfizer Australia Pty Ltd

24th July 2006

TABLE OF CONTENTS

SUMMARY

- Pregabalin is registered for the treatment of neuropathic pain and as adjunctive therapy in partial seizures with or without secondary generalisation. The new indication sought is for the treatment of generalised anxiety disorder (GAD).
- All but one of the submitted nonclinical studies had been previously submitted to the TGA. The new study, an *in vitro* safety pharmacology study investigating the effect of pregabalin on hERG currents, did not raise any safety concerns.
- Pregabalin displayed anxiolytic activity in a number of appropriate animal models, supporting its likely efficacy in GAD. Pregabalin also increased slow-wave sleep in animal studies.
- In August 2004 the sponsor received a non-approvable letter from the United States because of safety concerns regarding mouse carcinogenicity findings. Careful consideration of the carcinogenicity data in the original nonclinical report led to the conclusion that the observed mouse tumours were probably not relevant to humans, although this could not be entirely excluded. To date, the available data do not suggest that reconsideration of this conclusion is warranted.
- There are no nonclinical objections to the extension of indications for pregabalin to include GAD.

CONCLUSIONS AND RECOMMENDATIONS

In several appropriate animal models pregabalin showed anxiolytic activity, supportive of the proposed new indication of GAD. The draft Product Information document should be amended as indicated below. There are no nonclinical objections to the extension of indications for pregabalin to include GAD.

ASSESSMENT

Overall quality of the nonclinical dossier

The nonclinical studies submitted to support the proposed extension of indications to include GAD had been previously submitted with the original application for registration of pregabalin, but were re-submitted as the data were considered relevant to the current application. Only one of the submitted studies had not been previously submitted (PD144723/IC/001/05: an *in vitro* safety pharmacology study of the effect of pregabalin on hERG channels; see Section 4.2).

Efficacy

Pregabalin produced anxiolytic-like responses in the Vogel conflict test in rats s47 mg/kg PO, report 740-03464; s47 mg/kg PO, report 740-03576) and the Geller conflict test in rats s47 mg/kg SC, report 770-01316) and rhesus monkeys s47 mg/kg PO, report 740-03526). Two studies reported results for the elevated X-maze test in rats; in one of these pregabalin showed anxiolytic-like activity (ALA, s47 mg/kg SC; 770-01316) and in one it did not s47 mg/kg PO; 740-03464, Table 2.5). In the nonclinical overview (Module 2.4 p3) it is stated that an important difference between the two rat elevated X-maze tests was that one was conducted in low room light (770-01316, in which pregabalin showed ALA) and in the other higher room light was used (740-03464). However, this methodological detail was not available in the study reports, and so could

not be confirmed. The doses at which ALA was observed were similar to those that achieved analgesic and anticonvulsant activity. All of the tests described above are appropriate nonclinical models for anxiolytic activity, and these results give nonclinical support for the efficacy of pregabalin for the indication of GAD.

The sponsor has also claimed that pregabalin produced anxiolytic-like responses in the mouse tail-suspension test. However, this test is a behaviour despair paradigm used to assess potential antidepressant activity, and thus the inclusion of these test results is inappropriate in a list of nonclinical tests used to assess anxiolytic activity.

Secondary pharmacodynamics

There were no new pharmacology studies that could be classified as secondary pharmacodynamics. The previously submitted pharmacology studies related to anxiolytic activity were considered to be secondary pharmacodynamics when pregabalin was originally evaluated but, in view of the currently proposed extension of indications, are now appropriately classified as primary pharmacodynamics.

Safety Pharmacology

As noted in the previous nonclinical evaluation report, ataxia was observed in mice, rats, rabbits and monkeys, as well as being a common effect in humans.

A new *in vitro* study (PD144723/IC/001/05, section 3) showed that pregabalin at s47 µg/mL (*ca* ten times the C_{max} of s47 µg/mL in humans given s47 mg/day) decreased the peak hERG current amplitude by s47 but at s47 µg/mL pregabalin had little effect. There were no solubility issues with pregabalin, so higher concentrations could have been used, but given the small effect of pregabalin at concentrations ten times C_{max}, pregabalin is unlikely to prolong the QTc interval of the ECG.

Carcinogenicity

In August 2004 the sponsor received a non-approvable letter from the United States because of safety concerns regarding mouse carcinogenicity findings. These findings were considered carefully at the time of the initial application to TGA to register pregabalin, and were considered to be probably not relevant to humans, although this could not be entirely excluded. There are no new data to make relevance to humans more likely, and so there is no reason for the conclusion by the original nonclinical evaluator to be modified.

PRODUCT INFORMATION

The sponsor has proposed the following paragraphs, with proposed nonclinical changes from the statements in the currently approved document marked.

PHARMACOLOGY

Mechanism of action

"*In vitro* studies show that pregabalin binds to an auxiliary subunit (alpha$_2$-delta protein) of voltage gated calcium channels in the central nervous system, potently displacing ^{3}H-gabapentin. Two lines of evidence indicate that binding of pregabalin to the alpha$_2$-delta site is required for analgesic,

anxiolytic and anticonvulsant activity in animal models: (1) studies with the inactive *R*-enantiomer and other structural derivatives of pregabalin and (2) studies of pregabalin in mutant mice with defective drug binding to the alpha$_2$-delta protein. In addition, pregabalin reduces the release of several neurotransmitters, including glutamate, noradrenaline and substance P. The significance of these effects for the clinical pharmacology of pregabalin is not known.

"Pregabalin does not show affinity for receptor sites or alter responses associated with the action of several common drugs for treating seizures, anxiety or pain. Pregabalin does not interact with either $GABA_A$ or $GABA_B$ receptors; it is not converted metabolically into GABA or a GABA agonist; it is not an inhibitor of acute GABA uptake or degradation.

"Pregabalin prevents pain related behaviours in animal models of neuropathic and postsurgical pain, including hyperalgesia and allodynia.

"Pregabalin is also active in animal models of seizures, including maximal electroshock tonic extensor seizures in mice or rats, threshold clonic seizures from pentylenetetrazol, behavioural and electrographic seizures in hippocampal kindled rats and tonic and clonic seizures in DBA/2 audiogenic mice. Pregabalin does not reduce the incidence of spontaneous absence seizures in genetic absence epilepsy in rats from Strasbourg (GAERS).

"Pregabalin produces anxiolytic-like responses in the mouse tail-suspension test, the rat Vogel and Geller conflict tests, the rat elevated X-maze and rhesus monkey Geller conflict test. In addition, pregabalin administration increases spontaneous slow-wave sleep in rats and causes subtle ataxia, but does not prevent locomotion or consciousness in rats or mice."

It is recommended that the proposed new paragraph at the end of the "Mechanism of action" section be amended as shown below. It is not considered appropriate to include the results of the mouse tail-suspension test (a behaviour despair paradigm used to assess potential antidepressant activity) in a list of nonclinical tests used to assess anxiolytic activity. It is also recommended that the results of both studies that used the rat elevated X-maze test should be included (770-01316, 740-03464, Table 2.5). Ataxia was not only observed in mice and rats, but also in rabbits and monkeys (section 4.1).

"Pregabalin produceds anxiolytic-like responses in ~~the mouse tail-suspension test,~~ the rat Vogel and Geller conflict tests, in one of two ~~the~~ rat elevated X-maze studies and in the rhesus monkey Geller conflict test. In addition, pregabalin administration increases spontaneous slow-wave sleep in rats ~~and causes subtle ataxia,~~ but does not prevent locomotion or consciousness in rats or mice. Pregabalin caused ataxia in mice, rats, rabbits and monkeys."

MAIN BODY OF REPORT

1 INTRODUCTION

1.1 PROPOSED EXTENSION OF INDICATIONS

Pregabalin is currently registered for the treatment of neuropathic pain in adults and as an adjunctive therapy in adults with partial seizures with or without secondary generalisation. The new indication requested is for the treatment of generalised anxiety disorder (GAD) in adults.

Pregabalin treatment for GAD is recommended to start at 75 mg *bid*. Dosage may be increased to 300 mg per day after one week. Following an additional week the dosage may be increased to 450 mg per day; the maximum dosage of 300 mg *bid* may be achieved after an additional week. A similar escalating dosage regimen is recommended for the approved indications, and there is no change to the maximum recommended dose.

Only one of the submitted nonclinical studies (PD144723/IC/001/05; Section 4.2) had not been previously submitted to the TGA for evaluation.

1.2 CHEMISTRY AND FORMULATION

Pregabalin (code CI-1008, also PD 144723) is (S)-3-(aminomethyl)-5-methylhexanoic acid (structure shown in Figure 1.2.1). It has a mol wt. of 159.2 and is freely water soluble. Excipients are lactose, maize starch and talc, with the gelatin capsule containing titanium dioxide, sodium lauryl sulphate and colloidal silica.

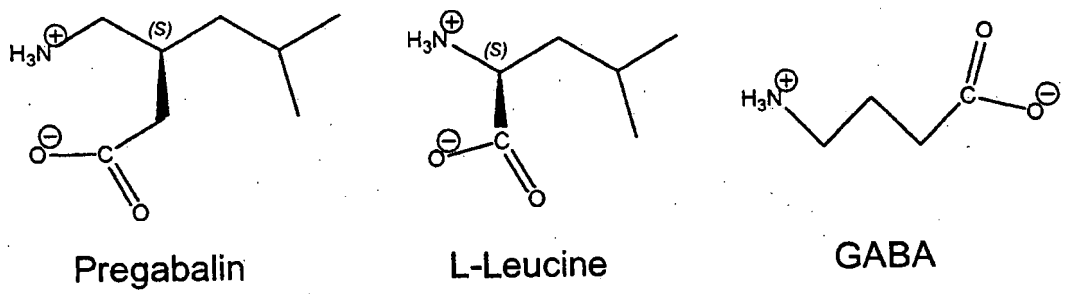


Figure 1.2.1 The structure of pregabalin and related compounds.

1.3 INTERNATIONAL STATUS AND REGULATORY HISTORY

An application for pregabalin for the treatment of GAD was submitted to Europe in June 2005 (centralised procedure, with the rapporteur as The Netherlands and the co-rapporteur as Portugal).

In August 2004 the sponsor received a non-approvable letter from the United States because of safety concerns regarding mouse carcinogenicity findings. These findings were considered carefully at the time of the initial application to TGA to register pregabalin, and were considered to be probably not relevant to humans, although this could not be entirely excluded.

1.4 RELATIONSHIP TO OTHER DRUGS

Gabapentin (1-aminomethyl cyclohexane acetic acid, Figure 1.4.1) can bind to $\alpha_2\delta$ subunits of recombinant voltage-gated Ca^{2+} channels (porcine type I and to a lesser extent human type 2) and pregabalin can also bind to these proteins as shown by displacement of bound [^{3}H]gabapentin, suggesting common receptors for both drugs.

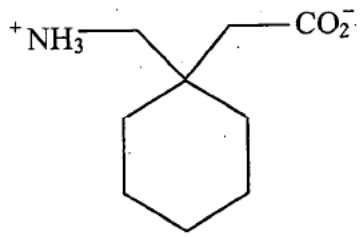


Figure 1.4.1 The structure of gabapentin

2 PRIMARY PHARMACODYNAMICS

In vitro studies showed that pregabalin binds to an auxiliary subunit ($\alpha_2\delta$ protein) of voltage gated calcium channels in the central nervous system, potently displacing ^{3}H-gabapentin (Table 2.1).

Table 2.1 In vitro radioligand binding studies

Report	Measurements/assays	Results
740-03239	Inhibition of [^{3}H]gabapentin binding to rat brain membranes.	Summary report. IC_{50} values (nM): 37 (pregabalin), 620 (R-enantiomer), 80 (gabapentin).
740-03172	Rat brain (membrane homogenate) [^{3}H]gabapentin radioligand binding	Published paper containing more pharmacodynamic data than 740-03239, but with the same IC_{50} values.
740-03602	Displacement of [^{3}H]gabapentin binding to porcine brain membranes, recombinant HEK cells expressing $\alpha_2\delta$-1 (porcine) and $\alpha_2\delta$-2 (human).	Respective K_i values (nM) for pregabalin, R-enantiomer, gabapentin: brain membranes: 19, 239, 28 $\alpha_2\delta$-1: 42, 480, 75 $\alpha_2\delta$-2: 44, 740, 114.
740-03603	[^{3}H]gabapentin binding to pig brain membranes, recombinant HEK cell membranes expressing $\alpha_2\delta$-1 (pig) and $\alpha_2\delta$-2 (human)#, brain membranes from R217A transgenic mice&.	K_d (nM): 20 (pig brain), 41 ($\alpha_2\delta$-1), 146 ($\alpha_2\delta$-2), 23 (mouse wild type), 53 (mouse heterozygous), 227 (mouse homozygous). B_{max} (pMol/mg): 0.92 (pig brain), 8.1 ($\alpha_2\delta$-1), 14.5 ($\alpha_2\delta$-2), 0.51-0.56 (mouse).
740-03576	Summarised below.	

#: $\alpha_2\delta$-1, $\alpha_2\delta$-2 = voltage-gated Ca^{2+} channel subunits 1 and 2; &: containing an Arg to Ala substitution at position 217 in $\alpha_2\delta$-1

In a composite study (**740-03576**), pregabalin and a series of structural analogues were tested for binding to pig brain membranes *in vitro* (competitive assay using [^{3}H]gabapentin) and for activity *in vivo* (in rat carrageenan thermal hyperalgesia, DBA/2 mice audiogenic seizure and rat Vogel conflict/anxiolytic tests). Treatment s47 [redacted] PO) was at s47 [redacted] prior to testing and there were significant correlations between *in vitro* binding (log IC_{50} values) and activity in all three *in vivo* tests. Respective pregabalin, racemate and R-enantiomer binding IC_{50} values were s47 [redacted] and s47 [redacted] nM.

Pregabalin reduced the release of several neurotransmitters, including glutamate, noradrenaline and substance P (Table 2.2).

Table 2.2 In vitro neurotransmitter release and turnover studies

Report	Measurements/assays	Results
770-00311	substance P facilitated/K^+-evoked glutamate release from rat brainstem slices (spinal trigeminal caudal subnucleus).	↓ by *ca* 75% with s47 µM pregabalin (only concentration tested) but not with R-enantiomer; ↓ 100% with 30 µM gabapentin.
740-03489	K^+-evoked release of [^{3}H]noradrenaline from rat neocortical slices; s47 mM tested.	↓ with maximal inhibition of 40%, IC_{50} for this inhibition range was s47 µM; corresponding values for gabapentin were 33% and s47 µM; ↓ with s47 µM pregabalin and 100 µM gabapentin were not additive.
740-03578	K^+-evoked/Ca^{2+}-dependent [^{3}H]neurotransmitter (noradrenaline, 5-HT, acetylcholine, dopamine) release from rat discrete CNS area slices; 100 µM tested.	Some ↓ release of all neurotransmitters (minimal for striatum dopamine); the largest ↓ was for neocortex noradrenaline and 5-HT (by 36-45%); identical results seen with gabapentin.
740-3579	Rat brain tissue slices (neocortex) [^{3}H]noradrenaline release in presence of gabapentin.	Pregabalin had no effect on [^{3}H]noradrenaline release in the presence of s47 µM gabapentin.
740-03470	Rat brain L-3,4-DOPA turnover & 5-HT turnover.	In rats pretreated with 3,4-diaminopyridine (a K^+ channel blocker) pregabalin dose-dependently ↓ release of L-DOPA but not 5-HT.
740-03538	K^+-induced Ca^{2+} influx in rat and cynomolgus monkey neocortical synaptosomes; s47 µM tested.	Slight ↓ in both preparations (by *ca* 18% vs 8% with the R-enantiomer).
740-03537	Capsaicin-induced release of immunoreactive substance P and calcitonin gene-related peptide from rat spinal cord slices; samples obtained contralateral and ipsilateral to an inflammatory stimulus (prior treatment with Freund's complete adjuvant).	Capsaicin elicited higher release of both mediators from ipsilateral (vs contralateral) slices which was ↓ by s47 µM pregabalin (by 50%); no effect of pregabalin on elicited release by contralateral slices.

The effects of pregabalin on the system L α-amino acid transporter and the GABA GAT1 transporter were investigated (Table 2.3). The system L α-amino acid transporter allows gabapentin as well as leucine and other large neutral α amino acids to cross the blood-brain barrier, but not GABA. The GABA GAT1 transporters are expressed in axons, presynaptic terminals and glial cells and are important in maintaining a low extracellular GABA concentration throughout the brain.

Table 2.3 In vitro transporter activity studies

Report	Measurements/assays	Results
761-00007	Rat brain cells (primary culture) or mammalian cell line [^{3}H]L-leucine uptake	Pregabalin competitively ↓ uptake of [^{3}H]-L-Leu into CHO cells (IC_{50} [redacted] µM). Intracellular pregabalin readily exchanged with extracellular leucine and gabapentin and [^{3}H]-pregabalin transport was blocked specifically by leucine and gabapentin. Pregabalin accumulated into cultured rat brain astrocytes, neocortical neurons, & cerebellar granule neurons.
740-03516	Effects of pregabalin on [^{3}H]GABA uptake and surface/intracellular localisation of the GABA GAT1 transporter protein (Western blot analysis) in neonatal rat hippocampal neurones.	Preincubation [redacted] x 2 h) ↑ GABA uptake and redistributed GAT1 transporter from the cytosol to the cell surface; EC_{50} [redacted] for ↑ GABA uptake (vs [redacted] µM for gabapentin) and it was apparent after ≥60 min preincubation; additive effect for ↑ GABA uptake with a protein kinase C inhibitor (bisindolylmaleimide II) and brain derived neurotrophic factor but not with Ω-conotoxin or nipecotic acid; pregabalin-induced ↑ GABA uptake abolished by a GAT1 inhibitor (SKF89976A) but botulinum toxin C1 had no effect.

Table 2.4 In vitro protein kinase activation studies

Report	Measurements/assays	Results
761-00006	Effects of pregabalin on MAP kinase signalling pathway in NK1-transfected CHO cells.	↓ substance P-elicited activation of transcription factor Elk-1 [redacted] additional studies carried out using gabapentin only.

Table 2.5 In vivo primary pharmacodynamic studies

Report	Measurements/assays	Results
740-03518	Electroshock seizure duration and paired-pulse inhibition in dentate gyrus of anaesthetised rats investigated; treatment was by IP injection.	Pregabalin ↓ pulse-paired inhibition [redacted] mg/kg) and ↓ lengthening of seizure discharge duration (maximal dentate activation, MDA) with repeated stimulation [redacted] kg); similar results obtained with gabapentin; nimodipine [redacted] mg/kg) ↓ MDA but slightly ↑ pulse-paired inhibition at [redacted] mg/kg.
740-03464	Rats: ALA: elevated X-maze, 10, 30, 100 mg/kg pregabalin PO and Vogel conflict test; [redacted] mg/kg pregabalin PO; PO treatment 2 h prior to testing. Mice: tail-suspension test (measures antidepressant activity) and the inverted screen test (measures coordination); [redacted] mg/kg pregabalin PO.	ALA demonstrated in the rat Vogel conflict test [redacted] mg/kg) but not the rat elevated X-maze test. In the tail-suspension test pregabalin-treated mice increased immobility and decreased power of movement, as did sertraline, an antidepressant and lorazepam, an anxiolytic, but not haloperidol, an antipsychotic (no effect on power of movement). Pregabalin-treated mice did not fall off the inverted screen.
740-03610	Mutant R217A mice& used; tail suspension period of immobility; period of licking/ biting after formalin injection (hyperalgesia) measured; [redacted] /kg PO dose tested.	Tail suspension test: ↑ immobility time (wild type) or no effect (homozygous). Hyperalgesia: ↓ licking time (wild type, heterozygous) or no effect (homozygous).
770-01316	ALA assessed in 2 rat models; SC treatment at 40 min prior to testing.	Elevated X-maze behaviour: ALA with [redacted] nd [redacted] kg of pregabalin. Geller conflict test with punishment: ALA with [redacted] /kg of pregabalin; slight ALA with 100 mg/kg of R-enantiomer.
740-03526	ALA assessed in a rhesus monkey punished response (Geller conflict) model.	↑ punished responses with PO pregabalin (30-300 mg/kg) consistent with ALA; similar results with PO alprazolam [redacted] /kg).
740-03551	Effect of flumazenil (benzodiazepine antagonist) on protection against audiogenic seizures in DBA/2 mice. ALA assessed in rats with Vogel conflict test at [redacted] /kg pregabalin PO.	Protection by 7 mg/kg PO of pregabalin and [redacted] mg/kg PO of gabapentin unaffected by [redacted] mg/kg IP of flumazenil; protection was also afforded by [redacted] /kg of PO diazepam, which was abolished by flumazenil. ALA in rats at [redacted] /kg; flumazenil did not antagonise these effects.

ALA: anxiolytic-like activity; &: containing an arginine to alanine substitution at position 217 in $\alpha_2\delta$-1

3 SECONDARY PHARMACODYNAMICS

Pregabalin did not show affinity for receptor sites or alter responses associated with the action of several common drugs for treating seizures, anxiety or pain. Pregabalin did not interact with either $GABA_A$ or $GABA_B$ receptors; it was not converted metabolically into GABA or a GABA receptor agonist; it was not an inhibitor of acute GABA uptake or degradation (Table 3.1).

Table 3.1 In vitro secondary pharmacodynamic studies

Report	Measurements/assays	Results
740-03076	Pregabalin and R-enantiomer s47 screened for receptor binding activity (38 radiolabelled ligand displacement assays).	No activity observed, including binding to $GABA_A$ and $GABA_B$ (rat), benzodiazepine (bovine), opioid (rat, guinea pig) receptors.
740-03547	Binding to recombinant human $GABA_B$ receptors using a ^{3}H-labelled GABA antagonist (CGP54626A); 2 cell lines used expressed the R1a/R2 or R1b/R2 receptor subtypes; rat cortical membranes also used.	No pregabalin or gabapentin binding; K_i values (μM) for GABA were 30.8 (R1a/R2), 29.5 (R1b/R2), 1.3 (brain membranes).
740-03548	Binding to recombinant human cannabinoid CB1 receptor and rat cortical membranes using a ^{3}H-labelled agonist (CP 55,940).	No pregabalin or gabapentin binding; K_i for CP 55,940 was s47 mM.
770-00350	Binding to human recombinant cannabinoid CB1 and CB2 receptors using [^{3}H]WIN 55212-2 ligand.	No pregabalin binding
740-03545	Affinity for neurotransmitter reuptake sites in rat brain synaptosomal preparations using ^{3}H-labelled dopamine, noradrenaline, 5-HT.	No pregabalin and gabapentin affinity.
761-00012	Effect on rat brain GABA transaminase activity.	No significant inhibition with s47 pregabalin or R-enantiomer; slight ↓ s47 gabapentin (by 32%).
760-00132	Effect on human platelet, U-937 cell and LPS-stimulated J774A cell cyclooxygenase activity; indomethacin positive control included.	No inhibition s47
740-03515	*Ex vivo* neonatal rat optic nerve GABA content at 150 min after IP treatment s47	No effect with pregabalin, gabapentin; ↑ GABA (>10x) with vigabatrin (GABA transaminase inhibitor).
740-03220	Voltage-clamped Na^+ currents in cells expressing recombinant rat brain type IIA Na^+ channels (α subunit).	No effect of s47 pregabalin; ↓ with 100 μM lidocaine.
740-03519	Voltage-gated ion channel currents in neonatal rat superior ganglion neurones, CHO cells expressing rat brain type IIA Na^+ channels (α_1 subunit), HEK cells expressing Ca^{2+} channels (human α_{1B}, rabbit muscle $\alpha_2\delta$, human neuronal β_2 subunit).	No ↓ s47 pregabalin.
740-03539	GABA-evoked currents in voltage-clamped rat fetal cortical neurones.	No effect of pregabalin (100 μM); ↑ with diazepam (EC_{50} = s47 and hexobarbitone (EC_{50} = s47
740-03517	Intracellular recordings in rat hippocampal slice CA1 region pyrimidal cells.	s47 pregabalin had no effect on NMDA or AMPA mediated synaptic transmission (epsp), GABA mediated inhibitory synaptic transmission (ipsp), long-term potentiation in stratum radiatum cells over 30 min.

4 SAFETY PHARMACOLOGY

4.1 CNS

The following reports (Table 4.1) were not resubmitted with this application, but the new wording in the proposed PI includes results from some of these studies. In particular, an increase in spontaneous slow-wave sleep in rats was demonstrated in report 740-03527. Pregabalin did not prevent locomotion or consciousness in rats or mice in any of these studies.

Ataxia has been observed in mice at 300 mg/kg IP (740-03224) and 300 mg/kg IV, plasma concentration 500 µg/mL (745-02928). In rats, ataxia was observed at 25 mg/kg PO (740-03224) and was also observed in safety pharmacology study 745-02928 (Table 4.1) and in various toxicity studies (1554, 1537, 1527, SP1645, 1960, 1962). In addition, ataxia was observed in both sexes in rabbits and monkeys (Table 4.2).

Table 4.1 CNS safety pharmacology studies

Report	Study type and experimental details	Results
745-02928 GLP-compliant	Neurofunctional evaluation in ♂ mice and rats; IV treatment with [redacted]; observation of clinical signs/behaviour over 60 min, at which time specific tests/measurements carried out (e.g. reflexes, co-ordination, grip strength, body temperature, locomotion).	Mice: urine staining/hypoactivity (HD), ataxia (1/8 HD), ↓ body temperature (LD, HD), ↓ vertical movements/ stereotypy time (LD, HD); 5 min plasma concs: 96 µg/mL (LD), 499 µg/mL (HD). Rats: hypoactivity (LD, HD), ataxia (1/8 HD), ↓ body temperature (LD, HD), ↓ vertical movements/stereotypy time/distance moved (LD, HD), ↑ tail analgesia (HD), ↑ foot splay distance (LD, HD); 5 min plasma concs: 104 µg/mL (LD), 591 µg/mL (HD).
740-03527	Sleeping monitored in ♂ rats with indwelling EEG, EMG and brain thermistor electrodes; PO treatment with [redacted] just prior to light cycle onset; recordings over 23 h.	↑ non-rapid eye movement sleep and EEG slow wave activity at [redacted], ↓ rapid eye movement sleep at [redacted] mg/kg, no change in sleep cycle length or latency to sleep; results considered consistent with hypnotic-like activity.
740-03215	Locomotor ataxia (gait/posture when walking), muscle tone, righting reflex assessed in ♂ rats at 2.5 h after PO treatment with [redacted] mg/kg.	↓ righting reflexes in 1/8 LD, 3/8 MD, 8/8 HD rats.
770-01317	♂ rat sensory motor co-ordination assessed in a beam walking test at intervals after PO treatment with [redacted] kg.	Marked ↑ crossing time and number of foot slips (all doses), ↑ rat falls (MD, HD); slight activity with 300 mg/kg gabapentin.
740-03217	♂ mice tested for ataxia using inverted screen test at 0.5-3 h after [redacted] or PO (1000 mg/kg) treatment.	No consistent effect of treatment.
740-03074	♂ mice tested for motor activity, and clinical signs observed, after IV [redacted] mg/kg) or PO [redacted] treatment.	↓ activity with IV and PO HD.
740-03472	♂ rats and mice tested for locomotor activity and ability to hang on to an inverted screen at 2 h after PO administration.	Rats: ↓ distance travelled at [redacted] kg, not seen with gabapentin, also apparent with lorazepam [redacted] ↑ falls in inverted screen test [redacted] not seen with gabapentin, also apparent with lorazepam [redacted] Mice: no significant effects on distance travelled with pregabalin or gabapentin [redacted] ↓ with lorazepam (≥1 mg/kg); no falls in inverted screen test with pregabalin or gabapentin [redacted] mg/kg), ↑ with lorazepam (≥3 mg/kg).
740-03224	Mouse rotorod ataxia test, IP treatment; Rat locomotion observation, PO treatment.	Mouse: ataxia in 2/8 at [redacted] ng/kg & 1/8 [redacted] kg. Rat ataxia dose-dependent ↑, with LOE [redacted] mg/kg; 75% ataxic [redacted] ng/kg.

Table 4.2 Rabbit and monkey toxicity studies in which ataxia was observed

Species	Sex	Study	Report no.	Route	Ataxia observed		No observed ataxia level
					Dose (mg/kg/day)	Plasma conc (µg/mL)	Dose (mg/kg/day)
Rabbit	♂	AA2644	745-03325	PO	s47 [redacted]	-	ND
	pregnant ♀	AA1907	745-02285	PO		-	ND
Monkey (cynomolgus)	Both sexes	1929	745-02329	PO		s47 [redacted]	s47 [redacted]
		1533	250-01720	PO		-	
		1916	750-02268	PO		-	
		2314	745-03033	IV		s47 [redacted]	ND
		SP1644	250-01817	IV (continuous infusion)			ND

ND: not determined; *C_{max}.

4.2 CARDIOVASCULAR TOXICITY

A new study was submitted (**PD144723/IC/001/05**, April 2005, GLP-compliant) in which a human embryonic kidney tumour cell line stably transfected with hERG channels was incubated with pregabalin (s47 [redacted] µM, 5 repeats). Standard techniques for whole cell patch clamping were used with 10 mM dofetilide as a positive control. There was no vehicle control conducted for the entire 15 min, but for the first 5 min when no pregabalin was present, the mean change in current amplitude was 5.8%. Pregabalin s47 [redacted] decreased the peak hERG current amplitude by s47 [redacted] and s47 [redacted] respectively, which when corrected for an expected background decrease of s47 [redacted] consisted of a possible drug-related effect of s47 [redacted] and s47 [redacted]

NONCLINICAL EVALUATION REPORT

FOR AN EXTENSION OF INDICATIONS

Application type:	Extension of indication
Sponsor:	Pfizer Australia Pty Ltd
Generic name:	**Pregabalin**
Trade name:	LYRICA®
Dose form and strength:	Capsules; 25, 50, 75, 100, 150, 200, 225 & 300 mg
Drug class:	Analgesic, Anti-epileptic
Submission No:	2006-0097-1
Tox file No:	2005/026478
Evaluator:	s22 [redacted]
Reviewer:	s22 [redacted]
Authorisor:	s22 [redacted]
Date:	**24th July 2006**

Note: This evaluation has been checked for confidential information and is cleared for release to the Sponsor only after this page is removed.