CENTER FOR DRUG EVALUATION AND RESEARCH

APPLICATION NUMBER:

022399Orig1s000

PHARMACOLOGY REVIEW(S)

Tertiary Pharmacology Review

By: Paul C. Brown, Ph.D., ODE Associate Director for Pharmacology and Toxicology, OND IO
NDA: 22-399
Submission date: 9/16/08; resubmitted 1/9/09 and 10/6/10
Drug: gabapentin encarbil
Applicant: GlaxoSmithKline
Indication: Restless Legs Syndrome (RLS)
Reviewing Division: Division of Neurology Products

Introductory Comments:
Gabapentin encarbil is an oral prodrug of gabapentin. A complete nonclinical development program was conducted for gabapentin encarbil. This information was generally complete and adequate; however, a carcinogenicity signal was identified in rats. Increases in pancreatic acinar cell hyperplasia, adenomas, and adenomas plus carcinomas were observed in rats. The increases were statistically significant in males and females at 5000 mg/kg/day. An non-statistically significant effect could not be ruled out at 2000 mg/kg/day in males. The dose of 500 mg/kg/day was associated with a plasma AUC in males that was only about 8 fold the human AUC at the clinical dose of 600 mg/day. There was inadequate information to establish that these tumors were not relevant to humans.

In the previous review of this NDA, the pancreatic tumors observed in the rat were a potential concern. A Complete Response letter was sent to the applicant on 2/17/10. The letter explained that the margin of exposure for the pancreatic tumor findings was unacceptable in the setting of RLS. The letter suggested some ways to address the concern such as demonstrating that the induction of pancreatic tumors in the rat occurs by a mechanism that does not occur in humans.

Discussion of new information:
The applicant provided several arguments in their resubmission as to why the tumor findings are not relevant to the human. They recalculated the exposure margins and suggested that the actual margin is larger than the 8-fold margin originally calculated. They also suggested that the pancreatic level of drug may be higher in rodents than humans, thus providing a larger margin in the pancreas as compared to the margin calculated with plasma levels of drug. The applicant also argued that the pancreatic tumors observed in rats are not relevant to humans based, in part, on the observation that in humans, pancreatic tumors primarily involve ductal, not acinar, cells.

The pharm/tox reviewer and supervisor have carefully reviewed the new information provided by the applicant. The data supporting the species differences in the level of drug in the pancreas was not considered convincing

because of potential limitations in the data and in the assays used to assess these levels. The relevance of rat acinar cell tumors to human pancreatic cancer remains unclear in part because of the uncertainty of the cellular origins of pancreatic tumors.

In the current submission, the applicant has referred to the Agency's finding of safety for gabapentin (Neurontin). Carcinogenicity studies with gabapentin showed the same tumor finding in rats, although only in males. The Agency considered 1000 mg/kg to be the no-effect dose for the occurrence of carcinoma. The applicant provided new data assessing the plasma exposure to gabapentin under conditions similar to those used in the original gabapentin rat carcinogenicity study in order to bridge to the exposures observed in the gabapentin enacarbil study. The exposure to gabapentin in the rat at 1000 mg gabapentin/kg was approximately 25 times higher than the human exposure and was intermediate between the exposures observed at 500 and 2000 mg gabapentin enacarbil/kg. This suggests that the NOAEL for the pancreatic tumors in rats actually occurs at an exposure to gabapentin that is 25-fold higher than the clinical exposure rather than the 8-fold margin at the 500 mg/kg dose.

Conclusions:
The pharm/tox reviewer and supervisor have conducted a thorough assessment of the new information provided by the sponsor. Although the mechanism by which the tumors are induced remains somewhat unclear and cannot be the basis for considering the tumors irrelevant to humans, the new information does permit an updated comparison of animal to human exposures at the NOAEL. This comparison suggests that the NOAEL for pancreatic tumors induced by gabapentin is approximately 25-fold higher than the human exposure. Carcinogenicity findings in rodents that occur at exposures greater than 25 times the human exposure are generally considered of limited human relevance and the risk:benefit analysis may now support the use of gabapentin enacarbil in RLS. Therefore, I agree with the division pharm/tox recommendation that the NDA can be approved from a pharm/tox perspective.

I also agree with the labeling suggestions from the pharm/tox supervisor.

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/s/

PAUL C BROWN
04/05/2011

MEMORANDUM

DEPARTMENT OF HEALTH & HUMAN SERVICES
Public Health Service
Food and Drug Administration

Division of Neurology Products (HFD-120)
Center for Drug Evaluation and Research

Date: April 5, 2011

From: Lois M. Freed, Ph.D.
Supervisory Pharmacologist

Subject: NDA 22-399 (XP13512, gabapentin enacarbil, HORIZANT®), Amendment N0045 (SDN 49), October 6, 2010

Background: the NDA for gabapentin enacarbil (HORIZANT) for treatment of moderate-to-severe Restless Legs Syndrome received a Complete Response (CR) action (Agency letter dated February 17, 2010) due to the pancreatic acinar cell tumors observed in the 2-year carcinogenicity study in rat (*cf. Pharmacology/Toxicology Review and Evaluation NDA 22-399, Terry S. Peters, D.V.M., 2/1/2010; Memorandum, NDA 22-399, Lois M. Freed, Ph.D., 2/5/2010)*, and also observed in a 2-year rat carcinogenicity study of gabapentin (*cf*. NEURONTIN labeling).

The sponsor (GSK) has submitted a complete response to the Agency's CR letter in Amendment N0045 (SDN 49), dated October 6, 2010. In this amendment, the sponsor has provided the following arguments to address the concern regarding the pancreatic acinar cell tumors observed in rat:

- The safety margin is greater than that calculated by the Agency, using "Three independent approaches".
- "There is a large body of evidence that rat pancreatic acinar cell tumors are not relevant to human disease."
- "The epidemiological data do not support a carcinogenic effect of gabapentin in humans."

The two nonclinical arguments and the supportive information have been reviewed by Dr. McKinney (*cf. Pharmacology/Toxicology NDA Review and Evaluation, NDA 22-399, April 5, 2011*) and are discussed in this memo. (For detailed discussion of the relevant data provided in the original NDA submission, see: *Pharmacology/Toxicology Review and Evaluation, 2010 by Terry S. Peters, D.V.M.; Memorandum for NDA 22-399, February 5, 2010, Lois M. Freed, Ph.D.*)

The original NDA was filed under 505(b)(1); however, the October 6, 2010 resubmission reclassifies the NDA as a 505(b)(2), with Neurontin as the Reference Listed Drug. Therefore, information for gabapentin is included in discussions and in labeling recommendations, as appropriate.

Complete Response (October 6, 2010)

- **Safety Margin**

The sponsor states that "It is acknowledged that the assessment provided in the [original] NDA was limited to a comparison of the carcinogenicity data for gabapentin enacarbil and gabapentin, which were completely consistent with each other, without any detailed discussion of safety margins."

[The original versions of labeling proposed by the sponsor (submitted 9/15/2008, 4/7/2009) provided safety margins; as stated in Section 13: "In a 2-year carcinogenicity study in rats, gabapentin enacarbil was given at doses of 500, 2,000, or 5,000 mg/kg/day by oral gavage. A significant increase in the incidence of pancreatic acinar adenoma and carcinoma was found at 2,000 or 5,000 mg/kg/day, which resulted in plasma exposures approximately (b) (4) times, respectively, the human gabapentin exposure at a recommended dose of 1,200 mg/day…"]

The sponsor notes agreement with the Agency's 8-fold safety margin, based on comparison of blood/plasma AUC (407 μg∗hr/mL) at the dose not associated with an increase in pancreatic acinar cell tumors (LD of 500 mg/kg/day) and the anticipated plasma AUC (51.4 μg∗hr/mL) in humans at the recommended human dose of 600 mg/day. However, the sponsor has now conducted "a more thorough examination" of the data from the 2-year carcinogenicity study of gabapentin enacarbil in rat and, "using three independent approaches", has concluded that "the safety margin for the proposed 600 mg clinical dose is 25-fold or greater.

Based on this new examination, the sponsor has concluded the following (taken directly from the sponsor's submission):

- The margin of safety for the proposed human exposure compared to the gabapentin enacarbil dose (2000 mg/kg/day) associated with a threshold response (a single carcinoma) in male rats is 38-fold.

- The margin of safety for the proposed human exposure compared to the gabapentin enacarbil dose with no carcinomas in rats (500 mg/kg/day) is >50-fold when corrected using new data on the species difference in pancreatic accumulation of gabapentin.

- The margin of safety for the proposed human exposure based on data from the published gabapentin carcinogenicity study at the no-effect dose (1000 mg/kg/day) is 25-fold. The use of these data to calculate the safety margin for gabapentin enacarbil was discussed with the FDA [NDA 022399, Sequence Number 0042, m1.6.3, Correspondence Regarding Meetings; Meeting Minutes]. At the suggestion of FDA, GSK has performed an independent toxicokinetic study in the same strain of rats using the same dosing methodology as the original gabapentin carcinogenicity study. This study was conducted to support the calculation of the safety margin associated with the no effect dose level for carcinoma.

- *New calculation of safety margin (gabapentin)*

Re-calculation of TK parameters: The sponsor provided revised estimates of plasma AUC data for the 2-year carcinogenicity data of gabapentin enacarbil based on new data in rat. In the original evaluation, the Agency had based the 8-fold exposure margin on TK data from the 6-month oral toxicity study in rat since it was considered the most relevant for estimating exposures achieved in the 2-year study. (Both studies were conducted using gavage administration.) In that study, TK analysis was conducted using whole blood. The sponsor conducted new 7- and 14-day dietary studies of gabapentin in rat (males only). In the 7-day study, TK data were analyzed using both whole blood and plasma. AUCs for gabapentin based on whole blood data were consistently lower (0.84-0.85 times) those based on analysis of plasma. The sponsor used these data to convert the whole blood AUC data from the 3-month (not the 6-month) oral toxicity study in rat to plasma AUC data. The sponsor chose the 3-month study "…because the 3 month study had more frequent sampling times…" (In the 6-month study, blood samples were collected at 1, 2, 4, 6, 10, and 24 hours post dose, whereas in the 3-month study, collection times were 0.5, 1, 2, 4, 8, 12, and 24 hours post dose.) The sponsor noted that this was particularly important for estimates of exposure at the LD since the T_{max} at that dose was 0.5 hours. (According to the TK data from the 3- and 6-month rat studies, the T_{max} in males at the HD ranged from 1 to 4 hours.)

The following table summarizes exposure (units of μg∗hr/mL) to gabapentin after oral administration of gabapentin enacarbil in male rats. Plasma data were calculated from whole blood data as follows: blood AUC/0.85 = plasma AUC.

STUDY	DOSE (mg/kg)	$AUC_{(0\text{-}inf)}$					
		BLOOD			PLASMA (estimated)		
		D1	D90	D175	D1	D90	D175
3-mo	500	393	474		462	558	
	2000	1590	1640		1870	1929	
	5000	2710	5620		3188	6612	
6-mo	500	382		470	449		553
	2000	780		1470	918		1729
	5000	4170		3230	4906		3800

The sponsor summarized the plasma exposure data for the 3-month gabapentin enacarbil study in the following table. The sponsor used the following equation to estimate plasma exposures from TK data collected using whole blood: plasma concentration (µg/mL) = 0.55 + 1.18 x blood concentration (µg/mL).

Table 8 Steady-State Toxicokinetic Parameters for Gabapentin Enacarbil Treated Rats

Parameter	Gender	Gabapentin Toxicokinetics (Plasma Levels)		
		500 mg/kg/day	*2000 mg/kg/day*	***5000 mg/kg/day***
$AUC_{0\text{-}24}$ (µg.h/mL)	Males	572	*1950*	***6640***
	Females	544	*1830*	***5870***
	Combined	558	*1890*	***6250***
C_{max} (µg/mL)	Males	154	*299*	***422***
	Females	180	*296*	***490***
	Combined	167	*298*	***456***

Key: ***Bold italics*** denotes dose level concluded to be carcinogenic.
Italics denotes dose level concluded to be threshold/slight effect level.
Based on correction for blood/plasma concentration differences.

One could argue with the sponsor's approach for estimating plasma exposure for gabapentin (i.e., using a conversion method determined only in males and with a different drug [gabapentin instead of gabapentin enacarbil] and dosing regimen [dietary instead of gavage]) and with using the 3-month (instead of the 6-month) TK data. However, the greatest difference is seen at the HD (plasma AUC almost 2-fold higher compared to blood AUC in the 6-month study), a dose that would not be the basis for establishing a safety margin since it is clearly an effect dose. At the LD, not clearly associated with an increase in pancreatic acinar cell tumors, the safety margin based on the new AUC estimates is ≈11 (vs 8-fold based on the original estimates).

Safety margin based on new historical control data: According to the sponsor, the historical control (HC) data for pancreatic acinar cell tumors provided in the original study report combined incidences for Wistar and Wistar:Han rats from the contract laboratory (b) (4)). Wistar:Han are reported to have a lower spontaneous rate of pancreatic acinar cell tumors than Wistar (the strain used in rat carcinogenicity study); therefore, the original HC data underestimated the spontaneous occurrence of pancreatic acinar cell adenoma and carcinoma. Based on the published incidence tables (compared with the new (b) (4) control dataset) for Wistar rat, the sponsor argues that "…the interpretation

would almost certainly have been that there was no carcinogenic effect at 2000 mg/kg/day [MD]…", resulting in a safety margin of 38, rather than 8-11. This is based on the fact that the sponsor considers the one carcinoma in the MD male to be consistent with the spontaneous rate, based on the new HC data.

In her review, Dr. McKinney discusses the HC data provided by the sponsor, and notes that due to the age of the studies (conducted prior to 1996), the lack of details regarding study methodologies, and, for the (b) (4) data, the lack of a sufficient number of control animals, the data are of limited value, particularly for making statistical inferences. Dr. (b) (4), who provided an expert opinion for the sponsor (*Expert Report* - (b) (4) M.D., Ph.D., August 12, 2010), recommends not making direct comparisons of tumor incidences from the gabapentin and gabapentin enacarbil carcinogenicity studies due to evolving differences in spontaneous lesions and responsiveness to "various stimuli" in test strains over time, noting that "studies with Gabapentin and GE were performed at markedly different years". This same reasoning is applicable to the lack of relevance of the published HC data provided by the sponsor.

As noted by the sponsor, interpretation of the data at the MD "…was very difficult…" Although the increase in pancreatic acinar cell adenoma and carcinoma was not statistically significant at the MD, the one carcinoma detected at that dose was fatal and, according to the Study Pathologist, there was also an increase in the incidence and/or severity of acinar cell hyperplasia at that dose; no acinar cell carcinoma was detected in any control animal (male or female) or in the (b) (4) HC data for Wistar rat. In addition, the MD and HD male groups were sacrificed early (97 and 90 weeks, respectively) due to increased mortality, possibly lessening the sensitivity for detecting additional preneoplastic or neoplastic findings in those groups. Taken together, these findings made it difficult to rule out a potential drug-related effect at the MD (*cf. Executive CAC meeting minutes*, 8/5/09). (As noted above, the sponsor's original proposed labeling stated that both the MD and HD were associated with increases in acinar cell adenoma and carcinoma.) Taking into consideration the new HC data, one of the three expert opinions (*Expert Report* - (b) (4), M.D., September 2, 2010) provided by the sponsor concluded that the findings at the MD represented "…the minimum possible response that could denote a carcinogenic response"; the other experts concluded that drug-related acinar tumors were evident only at the HD. None of the experts conducted a review of the original histopathology slides.

Safety margin based on species differences in pancreatic accumulation of gabapentin: The sponsor conducted two new studies in an attempt to determine whether or not interspecies differences in tissue uptake and distribution of gabapentin could explain species differences in sensitivity to gabapentin-induced tumors, and their relevance to humans. Specifically, the sponsor conducted (1) an *in vitro* study to assess the accumulation of gabapentin in pancreatic slices from rat and human and (2) an *in vitro* study to assess the expression and localization of gabapentin transporter proteins in pancreatic slices from mouse, rat, and human. These have been reviewed in detail by Dr. McKinney.

- Interspecies differences in gabapentin concentrations in pancreas.

The sponsor argues that previously published studies (Balkenohl M *et al*. *Epilepsia* 34:157, 1993; Radulovic LL *et al*., *Drugs Today* 31(8):P597-611, 1995a) and the new *in vitro* study assessing uptake of tritiated gabapentin into rat and human pancreatic slices demonstrate a rodent- (or species-) specific uptake of gabapentin.

The Balkenohl *et al*. (1993) data were published as an abstract; no full publication of these data could be found. The sponsor provided a copy of the poster presentation. According to the poster, gabapentin uptake was assessed *in vitro* in pancreatic tissue from 4 male Wistar Kyoto rats, 2 (1 M, 1 F) long-tailed green (Grivet) monkeys (>12 years old), and two human organ donors (25 and 52 years of age). Time from tissue collection to incubation was not stated for any species. After a 45-min incubation period, the pancreas-to-medium ratios for total radioactivity were 0.076, 0.125, and 1.960 for human, monkey, and rat tissue, respectively. In an *ex vivo* experiment in Grivet monkeys (1 M, 2 F) administered gabapentin orally at 50 mg/kg TID over 4 days, the ratio of pancreas-to-blood concentrations of gabapentin were approximately 1:1 at 4 hours after the last dose.

Radulovic *et al*. (Radulovic *et al*., 1995a; Radulovic LL *et al*. *Drug Met Disp* 23(4):441-448, 1995b) reported on the concentrations of gabapentin in mouse (strain unknown), Sprague-Dawley rat, and cynomolgus monkey pancreas following oral doses of 10, 10, and 25 mg/kg, respectively. Tissue concentrations of radioactivity were quantitated using whole body autoradiography (for rat, "classic excision and oxidation technique" was also used). At up to 4 hours post dose, pancreas-to-blood ratios were ≈8-10 in mouse, 5-7 in rat, and 1 in monkey. By 24 hours post dose, concentrations in the mouse and rat pancreas were at or below the LLOQ.

In the sponsor's study, accumulation of tritiated gabapentin was assessed using pancreatic tissue slices from 4 male Wistar rats and 3 male human organ donors. Incubation Time 0 was within 1 hour of surgical resection of the rat pancreas and within 1 hour of organ delivery to the laboratory for the human samples. (Time to organ harvest was 1 hr, but time from organ harvest to delivery was not specified.) The pancreas-to-medium (radioactivity) ratios at 0-3 hours were 1.85-1.80 for rat and 0.156-0.163 for human. The sponsor concluded that gabapentin accumulates at significantly higher levels in the pancreas of rat compared to human.

While these data suggest that gabapentin is distributed to a greater extent in rodent pancreas than in monkey or human, they are less than definitive. The Balkenohl *et al*. (1993) poster provides insufficient information to allow an evaluation, and the data were never published in full. The Radulovic *et al*. (1995a, 1995b) studies were conducted in an unknown strain of mouse and the Sprague-Dawley rat (not used in the carcinogenicity studies of gabapentin or gabapentin enacarbil) and, for the most part, used a fairly insensitive method of quantitation. The sponsor's study used the appropriate rat strain, but did not assess uptake into mouse pancreas; regarding the human data, the relatively long delay in tissue preparation could account for, or at least contribute to, the substantially lower uptake of gabapentin into human tissue. (The use of total radioactivity

data in the *in vivo* studies is acceptable since gabapentin is metabolized *in vivo* only to a limited extent.)

- Interspecies differences in expression and localization of gabapentin transport proteins.

The sponsor conducted a study using pancreatic tissue from male Wistar rat, male Sprague-Dawley rat, male B6C3F1 mouse, and human organ donors (76 year old female, 83 year old female, 44 year old male). Gabapentin transport was assessed *in vitro* for 29-43 solute carrier transporters, 6 of which are gabapentin transporters (LAT1/4F2hc, LAT3, LAT4, OCTN1, OCTN2, OCT2). (4F2hc is a heavy chain binding partner, required for trafficking of LAT1 to the cell surface.) Of these, the LAT1/4F2hc transporter demonstrated the highest affinity for gabapentin (for the human transporter, $K_M = \approx 200\ \mu M$); affinity for other gabapentin transporters was at least 10-fold lower. Staining for LAT1/4F2hc transporter gene expression in mouse, rat, and human pancreatic tissue indicated that:

  - Staining in human pancreas was primarily observed in islet cells, with lower amounts detected in acinar cells.
  - Staining in Wistar rat pancreas exhibited higher ("striking") levels in acinar cells, and lower levels in islet cells.
  - Staining patterns for Lat1 in pancreas of Sprague-Dawley rat and B6C3F1 mouse were similar to those for Wistar rat.

The data for selected transporters were summarized in the following sponsor's table (LAT2 was stated to "not recognize gabapentin"):

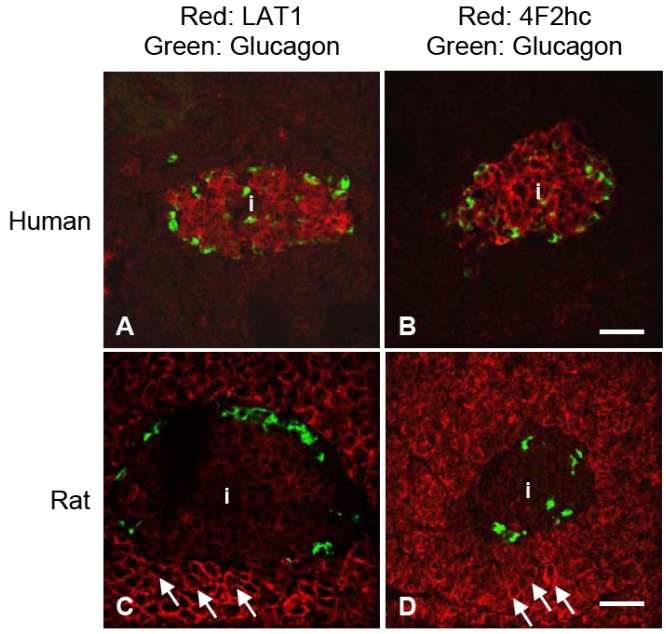
Table 12 Comparison of Human and Wistar Rat Expression of SLC Transporters in Pancreas Normalized to the Housekeeping Gene GAPDH (1,000,000 copies) [XD2010/00358/00]

Transporter	Human Average (n=3)	Rat Average (n=4)	Expressed Ratio Rat vs. Human	Mouse Average (n=4)
LAT1	31,178	418,796	**13.4**	505,490
LAT2	18,757	881,669	**47.0**	1,156,642
LAT3	5,799	7,142	**1.2**	16,501
LAT4	653	18,910	**29.0**	59,337
4F2hc	50,943	1,435,724	**28.2**	367,889
OCT2	9,305	NS[a]	---	1,172
OCTN1	8,107	5,380	**0.7**	7136
OCTN2	14,895	26,439	**1.8**	31,618

Key: a = Not expressed.

Staining patterns for LAT1 and 4F2hc in human and rat pancreas are illustrated in the following sponsor's figure:

Figure 4 Immunofluorescence Staining of LAT1 and 4F2hc in Human and Rat Pancreas



In human pancreas, LAT1 (red, A) and 4F2hc (red, B) are detected primarily in islets (marked in the center with the letter i), defined by glucagon-positive alpha cells (green, A-B) around the islet periphery. In rat pancreas, staining for LAT1 (red, C) and 4F2hc (red, D) was stronger in acinar cells (examples indicated by arrows), and was less intense in islets (marked in the center with the letter i), identified by glucagon staining (green, C-D). Scale bars: 50 μm.

The sponsor states that these new data indicate that gabapentin is unlikely to accumulate in the pancreas in humans, although acknowledging that "…no data exist on human tissue distribution of gabapentin in vivo…" The sponsor also acknowledges that gabapentin accumulates in mouse pancreas, but notes that pancreatic tumors may not have been observed because (1) "…the gabapentin systemic exposures [and, thus, pancreatic tissue levels] in the mouse carcinogenicity study were lower than those examined in the rat gabapentin enacarbil carcinogenicity study and (2) "…the mouse has a much lower propensity for pancreatic tumor cell formation in the presence of a stimulus…"

Using these data, the sponsor states that "Any safety margin calculated purely on the basis of systemic exposure would substantially underestimate the true difference in target tissue exposure, potentially by a factor of >5-fold, thus suggesting a safety margin of >50 at the LD used in the rat carcinogenicity study. However, in summarizing plasma exposure ratios for pancreatic acinar cell tumors, the sponsor does not increase the safety margins to take into account interspecies differences in pancreatic tissue uptake (sponsor's table, below):

Table 17 Exposure Ratios for Gabapentin-Related Pancreatic Acinar Cell Tumors in Rats (Data ordered by AUC)

Gabapentin Enacarbil					Gabapentin		
Gabapentin Enacarbil Dose (mg/kg/day)	Gabapentin AUC (μg.h/mL)		Exposure Ratios		Gabapentin Dose (mg/kg/day)	Gabapentin AUC (μg.h/mL)	Exposure Ratios**
	Male	Female	Male	Female			Male
5000	***6640***	***5870***	***129X***	***114X***			
2000	*1950*	1830	*38X*	36X	2000	***1780[b]***	35X
					1000	1300[b]	25X
500	572	544	11.1X*	10.6X	250	515[a]	10.0X
0	0	0	-	-	0	0	-
600 mg clinical	51.4						

Key: ***Bold italics*** denotes clearly carcinogenic. *Italics* denotes threshold/slight effect.
Data are ordered by AUC of ~500 μg.h/mL increments.
Toxicokinetic data and clinical data [XP081] are from steady-state plasma levels.
* This margin is slightly higher than the value cited in the complete response letter. This margin was calculated based on the 3 month rat study and was corrected for blood to plasma concentration differences.
** Data from Radulovic (1995b) are gender combined, while data from 2010N105806_00 are for males only (blood data converted to plasma).
a = Radulovic, 1995b (combined gender data). b = 2010N105806_00.

These data demonstrate greater expression of gabapentin transporters in pancreas of mouse and rat than in human pancreas. However, the distribution of expression of LAT1 (the transporter with the greatest affinity for gabapentin) was different in rodent and human; in human pancreas, expression was concentrated in islet cells. As Dr. McKinney points out, these data suggest that gabapentin may concentrate in islet cells in humans, possibly to a similar extent as in pancreatic acinar cells in the rodent. Thus, using total concentrations of gabapentin in the pancreas may not be the most relevant parameter and, therefore, increasing the safety margin by some factor based on differences in total concentration may not be warranted. (It is unclear exactly on what the 5-fold factor is based.)

Overall, the new data on blood/plasma ratios and gabapentin transporters provided by the sponsor do not, alone, provide a clear basis for adjusting the safety factor for pancreatic acinar cell tumors. However, the results of the sponsor's 14-day dietary study of gabapentin in rat may provide a basis for re-evaluating this issue.

In the 14-day dietary study, doses of 1000 and 2000 mg/kg/day were associated with plasma AUCs of 1300 and 1780 ng∗hr/mL. These exposures were similar to those reported in a published bridging dietary TK study conducted by the Neurontin sponsor (Parke-Davis Pharmaceuticals) (Radulovic LL *et al*. *Drugs Today* 31(8):P597-611, 1995); plasma AUCs of ≈1250 and 2050 at 1000 and 2000 mg/kg/day were estimated from a figure presentation of the data (Figure 4).

The results of the 2-year dietary carcinogenicity study of gabapentin were published by Sigler *et al*. (Sigler RE *et al*. *Toxicology* 73-82, 1995). The incidences of pancreatic acinar cell tumors in this study are summarized in the following table (from *Memorandum, NDA 22-399, Lois M. Freed, Ph.D., 2/5/2010*).

FINDING	DOSE (mg/kg)			
	0	**250**	**1000**	**2000**
hyperplasia	21/50	22/50	20/50	23/50
adenoma	7/50	6/50	10/50	16/50
carcinoma	0/50	4/50	3/50	8/50

As evident from the table, there was a dose-related numerical increase in pancreatic adenoma + carcinoma across treatment groups. It is notable that, although the increase in carcinoma was not dose-related between 250 and 1000 mg/kg/day, the incidence in LD and MD groups (as well as at the HD) was greater than in control males; no carcinoma was reported in control males. One of the publications (Walsh KM, Poteracki J *Fund Appl Toxicol* 22:65-72, 1994) cited by the sponsor provided HC data from 10 carcinogenicity studies conducted in Wistar rat between 1980 and 1990. These studies were conducted by Parke-Davis, and may have included the control data from the 2-year carcinogenicity study of gabapentin. Therefore, the spontaneous incidence of pancreatic acinar cell carcinoma reported by Walsh & Poteracki (1994) should be relevant HC data for the gabapentin study. Walsh & Poteracki (1994) report an incidence of 0.6% (range: 0-2.0). Using the highest HC incidence reported, one carcinoma in 50 male rats or two carcinoma in 100 male rats might be expected; therefore, the incidence in LD and MD male groups substantially exceed the reported spontaneous rates.

However, based on Neurontin labeling, the mid-dose (1000 mg/kg/day) was determined to be the highest dose tested that was not associated with an increase in pancreatic acinar cell tumors. The labeling states

> "A statistically significant increase in the incidence of pancreatic acinar cell adenomas and carcinomas was found in male rats receiving the high dose; the no-effect dose for the occurrence of carcinomas was 1000 mg/kg/day."

Without a better understanding of what informed that decision, the MD is accepted as a "no-effect" dose for gabapentin.

Using the bridging TK data provided by the sponsor, the 1000 mg/kg/day dose would provide an ≈25-fold safety margin for the 2-year rat carcinogenicity study of gabapentin, based on plasma exposure. This, then, would be a basis for increasing the safety margin for gabapentin enacarbil, from 8-10 to ≈25-fold.

- **Relevance of rat pancreatic acinar cell tumors to human disease**

The sponsor has addressed this issue in several ways, one or more of which have been discussed in relationship to estimates of the safety margin. The sponsor argues that

pancreatic acinar cell tumors in rat are species-specific and not relevant to humans, based on the following:

- Rat is uniquely sensitive to gabapentin-induced pancreatic acinar cell tumors, based on greater uptake of gabapentin into the pancreas and the "unusually high" spontaneous rate of these tumors in rats compared to humans.
- The male rat is particularly sensitive to gabapentin-induced pancreatic acinar cell tumors due to differential effects of male and female sex hormones.
- "The difficulty in definitively proving a mechanism for gabapentin to stimulate formation of rat acinar cell tumors."
- Gabapentin is non-genotoxic.
- "The lack of a general mitogenic effect for gabapentin."
- Pancreatic acinar cell tumors are spontaneous findings in rat, whereas in human, the majority of pancreatic tumors are ductal adenocarcinomas.

Each of these will be discussed briefly, except for the lack of a genotoxicity signal for gabapentin, which has already been demonstrated; it is accepted that gabapentin exerts its tumorigenic effects through an epigenetic mechanism.

In support, the sponsor conducted one mechanism-of-action study (an *in vivo* CCK study) and provided three expert opinions ([redacted] (b) (4)

Unique sensitivity of rat to gabapentin-induced pancreatic acinar cell tumors: The sponsor argues that the rat, "especially male Wistar rats", is uniquely sensitive to gabapentin-induced pancreatic acinar cell tumors, as evidence by "unusually high" spontaneous rates compared to humans. The sponsor reports spontaneous rates of 3.1-17, 0.4-1.8, 0-0.7, and 0-0.1% in male Wistar rat, male non-Wistar rat, female Wistar and non-Wistar rat, and male and female mice, respectively. In humans, "pancreatic acinar cell tumors are exceedingly rare…with an annual age-adjusted incidence of less than 1.17 per million persons, based on an NCI report (*SEER Cancer Statistics Review 1975-2006*). Issues regarding the HC data provided by the sponsor to support the increased spontaneous rates on Wistar vs Wistar-Han have been discussed previously. In addition, as noted by Dr. McKinney, the relatively high rate reported by the sponsor for male Wistar rat (mean of 13.3%) was based on an analysis by Poteracki & Walsh (Poteracki J, Walsh KM *Toxicol Sci* 45:1-8, 1998). According to that publication, of the 5 studies (conducted 1990-1995) included in the analysis, one had unusually high incidences of both pancreatic acinar cell adenoma and carcinoma; if this outlier study were removed from the analysis, the spontaneous rates in Wistar rat would be similar to those obtained in a previous publication (Walsh & Poteracki, 1994), i.e., 2.5 and 0.3% for adenoma in male and female, respectively, and 0.6 and 0% for carcinoma in male and female, respectively.

While the sponsor did not provide adequate relevant HC data, the available data suggest that in female Wistar rat, pancreatic acinar cell tumors, particularly carcinoma, are rare. In concurrent controls, no carcinoma was detected in 60 control males or 60 control

females; in addition, only 2 adenoma were detected in the 120 control animals (2 males). Based on these data, the fact that gabapentin enacarbil clearly produced an increase in pancreatic acinar cell adenoma/carcinoma in female Wistar rat would argue against the notion that gabapentin enacarbil (gabapentin) induced tumors only in a sensitive species, i.e., one in which the spontaneous rate of these tumors is high.

Increased sensitivity of the male rat to gabapentin-induced pancreatic acinar cell tumors: Addressing the lower effect-dose for gabapentin enacarbil in the male rat, the sponsor argues that the male Wistar rat, compared to the female Wistar rat, has a higher spontaneous rate of pancreatic acinar cell tumors and, therefore, is inherently more sensitive to gabapentin-induced pancreatic acinar cell tumors. The sponsor provides two published references to document differential effects of male and female reproductive hormones on induction of pancreatic acinar cell tumors (Longnecker DS, Sumi C *Int J Pancreatol* 7:159-165, 1990; Sumi C *et al*. *Cancer Res* 49:2332-2336, 1989). Sumi *et al*. (1989) reported that estradiol and castration inhibited the incidence of acidophilic atypical acinar cell foci and nodules (AACN, a precursor to pancreatic acinar cell tumors) induced by azaserine (a direct-acting mutagen) in Fischer rats. In intact animals, the authors note that the incidence of AACN was significantly higher in males than in females and that these data are consistent with previous studies of the role of sex hormones (particularly male sex hormones) on pancreatic acinar cell tumors. These data suggest that male sex hormone(s) may underlie the higher spontaneous rate of pancreatic acinar cell tumors and the apparently greater sensitivity to factors inducing these types of tumors in males. However, as pointed out by both Sumi *et al*. (1989) and Longnecker & Sumi (1990), "In epidemiologic studies, the age-adjusted incidence of pancreatic cancers is higher in males than in females by a factor of about 1.6:1" and "Slightly longer average survival has been reported in female than in male pancreas cancer patients…" (quotes from Longnecker & Sumi, 1990). Longnecker *et al*. (Longnecker DS *et al*. *Yale J Biol Med* 65:457-464, 1992) state that "In rats and mice but not in hamsters, exocrine carcinomas have a higher incidence in male than in female animals, as is true in the human." It is also of note that much of the cited studies regarding sex-related differences in pancreatic carcinogenesis have been conducted in the rat (*cf.* Longnecker & Sumi, 1990). Therefore, these data support neither the notion that the pancreatic acinar cell tumors in male rats are not relevant to human nor an adjustment to the safety margin.

Mechanism of action underlying pancreatic acinar cell tumors: The mechanism by which gabapentin induces pancreatic acinar cell tumors in rats is unknown. One proposed mechanism for pancreatic acinar cell tumors resulting from administration of other compounds (e.g., raw soy flour, casein, corn oil) is compound-induced prolonged elevations in cholecystokinin (CCK). Since rat pancreas is considered particularly sensitive to CCK-mediated effects, data demonstrating that gabapentin enacarbil might induce pancreatic acinar cell tumors by increasing circulating levels of CCK might mitigate the concern for humans. Dethloff *et al*. (Dethloff L *et al*. *Toxicol Sci* 55:52-29, 2000) stated that an effect of gabapentin on CCK could not be detected in preliminary studies in Wistar rat (cf. de la Iglesia *et al*. *The Toxicologist* 36, 905a, 1997; this citation could not be found), but noted that "…assay methodology…may be problematic…"

The sponsor conducted a pilot study in male Wistar rats. Gabapentin enacarbil was administered as a single 5000-mg/kg dose (the HD in the carcinogenicity study) by oral gavage; blood samples were collected up to 120 min post dose. No increase in CCK was obtained. Neither casein nor corn oil (positive controls), tested at a single dose of 5000 and ≈10,000 mg/kg, respectively, demonstrated a substantial increase in CCK; casein exhibited the greatest response (2.5 fold over baseline), but the mean baseline level in the casein-treated animals was also lower than in the other groups (including control). Based on these preliminary findings, the sponsor concluded that it would not be possible to demonstrate that gabapentin enacarbil increases CCK, "Based on the limitations of the RIA assay and the small magnitude of the differences…"

Dethloff *et al*. (2000) also investigated the mitogenic potential of gabapentin in order to determine whether or not gabapentin was a tumor promoter, increasing the incidence of pancreatic acinar cell tumors by stimulating cellular proliferation. No evidence of pancreatic acinar cell proliferation was detected in Wistar rats fed gabapentin (2000 mg/kg; the HD used in the carcinogenicity study) in the diet for up to 274 days; however, evidence of cellular proliferation was obtained in *in vitro* studies. The authors concluded that:

> "…its ability to increase intracellular calcium suggests that gabapentin may activate postreceptor downstream effectors and trigger proliferative signaling pathways. Using incorporation of ^{3}H-thymidine as an indicator of S-phase activity and cell proliferation, the *in vitro* data support the notion that gabapentin may stimulate DNA synthesis in normal pancreatic acinar cells. Concentrations at which gabapentin stimulated ^{3}H-thymidine incorporation in normal acinar cells are comparable to the plasma concentrations of approximately 110 µg/ml, associated with increased pancreatic acinar cell tumors in rats…Acting through this mitogenic pathway, gabapentin may behave as a weak tumor promoter…"

This conclusion is reflected in Neurontin labeling, which notes that "It is not known whether gabapentin has the ability to increase cell proliferation in other cell types or in other species, including human." The sponsor conducted no additional studies to further investigate the mitogenic potential of gabapentin enacarbil. The sponsor concluded, based on organ weights and histopathology that there was no evidence of a mitogenic effect in the chronic toxicity studies of gabapentin enacarbil in rat and monkey. However, cellular proliferation was not directly assessed in these studies, and the durations of dosing may not have been sufficient to detect an effect on cellular proliferation if prolonged exposure is necessary (as suggested by the lack of BrdU incorporation after 274 days of dosing in the *in vivo* study).

Therefore, *in vitro* data suggest that gabapentin acts as a mitogen. The role of mitogenicity in tumor formation is well recognized (*cf.* Alberts B *et al. Molecular Biology of the Cell*, 4th edn. New York:Garland Science, 2002; Cohen SM, Ellwein LB *Science* 249(4972:1007-1011, 1990; McQueen CA *et al*. *Comprehensive Toxicology*, 2nd edn. Vol 14, United Kingdom:Elsevier Ltd., 2010). Based on the limited data available, it is not possible to definitively characterize gabapentin as a "weak" mitogen.

Lack of correspondence between pancreatic acinar cell tumors and human pancreatic tumors: The sponsor argues that in humans, pancreatic tumors primarily involve ductal, not acinar, cells, and that, therefore, gabapentin enacarbil-induced acinar cell tumors are not relevant to humans. Drs. (b) (4) both address this issue and both support this view. Certainly, there is sufficient published literature to document the difference between human and rat in terms of the cellular characteristics of pancreatic tumors. Dr. (b) (4) notes that pancreatic acinar cell carcinomas account for <1% of pancreatic neoplasms in humans (*Expert Report* - (b) (4)

However, there is an increasing appreciation of the complexity of the pancreas and the plasticity of pancreatic tissue, particularly relating to the cellular origins of pancreatic tumors, which Dr. McKinney discusses in her review. Briefly, the cell of origin for pancreatic tumors in humans is unknown (Fendrich V *et al*. *Gastroenterol* 135:621-631, 2008; Habbe N *et al*. *Proc Natl Acad Sci* 105(48):18913-18918, 2008; Pour PM *et al*. *Mole Cancer* 2:13-22, 2003; Stanger BZ, Dor Y *Cell Cycle* 5(1):43-46, 2006). In the pancreas, acinar, ductal, and islet cells all arise during embryonic development from the same progenitor cell. However, according to Stanger & Dor (2006), stem cells have not yet been successfully isolated from adult pancreas and "…their existence is questioned." Processes (dedifferentiation, transdifferentiation, and reprogramming) by which mature cells can transition to other cell types contribute to regeneration and repair (and possibly to tumor formation) in various organs, including the pancreas (Jopling C *et al*. *Nat Rev Mole Cell Biol* 12:79-89, 2011). Transdifferentiation is a process in which adult cells change phenotype with or without regression to an intermediate progenitor-like state. A number of published studies have investigated the possibility that pancreatic ductal adenocarcinoma, the pancreatic neoplasm "most feared by the human population" (*Expert Report* - (b) (4), August 12, 2010), may arise by transdifferentiation of other pancreatic cell types. In *in vitro* and/or *in vivo* studies, transdifferentiation of pancreatic acinar cells into insulin-producing β cells, acinar cells into cells with a duct-like phenotype, β cells into acinar or ductal cells, ductal to islet cells have been reported. Indeed, Pour *et al*. (2003) conclude that "…all pancreatic cells could be considered as a potential facultative stem cell." According to Husain & Thrower (Husain S, Thrower E *Curr Opin Gastroenterol* 25:466-471, 2009), "Mounting evidence suggests that pancreatic ductal adenocarcinoma…and its noninvasive precursor lesion known as pancreatic intraepithelial neoplasia…are the result of acinar cell metaplasia to a ductal cell form." However, other investigators have proposed other cell types, e.g., islet cells (Jamal A-M *et al*. *Cell Death Differentiation* 12:702-712, 2005; Pour PM *et al*., 2003) or centroacinar cells (Stager & Dor, 2006), as the origin of ductal adenocarcinomas. Habbe *et al*. (2008) note that "Because carcinogens can adversely impact multiple cell types in the pancreas, the lineage fidelity from cell-of-origin to eventual neoplasia is hard to preserve…" Whether or not effects on acinar cell (or other "progenitor" cell) proliferation could give rise to, for example, acinar cell tumors in one species and ductal adenocarcinoma in another is unclear.

Dr. (b) (4) also points out that an activating mutation in *Kras* oncogene is prevalent in pancreatic ductal adenocarcinoma in humans, but not in human acinar carcinoma. It is

well recognized that mutated *Kras* is expressed in and contributes to a substantial number of human tumors, including tumors of the pancreas (De La O J-P *et al*. *Proc Natl Acad Sci* 105(48):18907-18912; Kranenburg O *Biochim Biophys Acta* 1756:81-82, 2005). According to Dr. (b) (4) this suggests that "…acinar and ductal carcinoma have different causes and pathways of development", and that pancreatic acinar cell tumors in humans have limited (if any) relevance to human. However, as Dr. (b) (4) notes, human acinar carcinomas do not express mutated *Kras*; neither does the rat pancreatic acinar cell tumor. Therefore, the argument that the rat tumors are not relevant to humans based on presence of mutated *Kras* does not apply to human acinar carcinomas. In addition, data for human pancreatic cancer suggest that, although *Kras* mutations may be an early event, other signaling pathways may be involved (e.g., Notch); human pancreatic tumors are polyclonal and are characterized by "a number of genetic alterations" (Pour *et al*., 2003).

- **Conclusions**

To address the pancreatic acinar cell adenoma and carcinoma observed in the lifetime carcinogenicity study of gabapentin enacarbil (and of gabapentin) in rat, the sponsor provided limited new data and three expert opinions. Several approaches were used in an attempt to reduce concern regarding the human relevance of these tumors; two were nonclinical. The sponsor argued that the safety margin is higher than the 8-fold originally estimated (based solely on the sponsor's data) and that the pancreatic acinar cell tumors in rat are not relevant to human.

In my opinion, the sponsor did not establish the lack of human relevance of the pancreatic tumors in rat. The sponsor argued for lack of human relevance based on several factors, including: (1) in rat, the tumors were of pancreatic acinar cells, whereas in human, the majority of pancreatic tumors are of the ductal type and (2) pancreatic acinar cell tumors are common (or not uncommon) spontaneous findings in rat, but not in human. Published studies suggest that the actual cell of origin of pancreatic tumors in animals and human remains uncertain and that all pancreatic (acinar, ductal, islet) cell types have the potential to give rise to cells of a different phenotype within the pancreas (e.g., acinar to ductal, islet to ductal). In this respect, the sponsor's data demonstrating the greater presence of a high-affinity gabapentin transporter (LAT1) in human pancreatic islets is of interest. These data suggest that gabapentin may concentrate in islet cells in humans and acinar cells in rat. Considering the "remarkable degree of morphogenetic plasticity" reported for adult human islets (Jamal *et al*., 2005; also Pour *et al*., 2003) and the mitogenic potential of gabapentin, this could potentially lead to pancreatic tumors (of one or more phenotype) in humans. Dr. (b) (4) (Pettengill OS *et al*. *Am J Path* 143(1):292-303, 1993) states that acinar cells comprise ≈80% of the pancreas; however, Pour *et al*. (2003) suggest that estimates that exocrine cells (acinar, ductal) make up 95% of the volume of the pancreas may be misleading and that "…it appears that there are as many islet cells as acinar cells."

The sponsor argued that the rat (particular the male) is more sensitive than human to drug-induced pancreatic tumors, based on interspecies differences in the spontaneous

rates of pancreatic acinar cell tumors and demonstrated responsiveness of rat pancreas to various agents (e.g., trypsin inhibitors, corn oil, asazerine). However, gabapentin enacarbil produced a clear tumorigenic effect in female rat and spontaneous incidence data consistently demonstrate that pancreatic acinar cell tumors are rare in female rats. According to the three sources of HC data cited by the sponsor, spontaneous rates of such tumors in Wistar rat range from 0 to 0.6% for adenoma and 0% for carcinoma. The greater spontaneous incidence rate in male (vs female) Wistar rat has been attributed to differential effects of sex hormones (protective for female and facilitative for male sex hormones) on development of pancreatic acinar cell tumors. A similar gender susceptibility has been reported for humans (estimated to be 1.6-2.0:1, M:F), suggesting that sex hormones may also affect development of pancreatic tumors in humans.

Pancreatic acinar cell tumors have been produced in rodents treated with genotoxic and non-genotoxic compounds (e.g., corn oil, raw soy flour, trypsin inhibitors, and peroxisome proliferators [e.g., clofibrate]), thus, according to the sponsor, demonstrating an increased sensitivity to induction of pancreatic tumors compared to human. How this alone mitigates concern for humans is not entirely clear, since interpretable data on the carcinogenic effect of compounds in humans are difficult to obtain.

A comprehensive review of published studies on these complex issues is beyond the scope of this memo. However, regardless of whether or not plasticity of pancreatic cells may affect the relevance of the rat tumor findings to human, published literature does demonstrate the lack of a full understanding of the processes involved in regeneration and cellular proliferation within the pancreas. This, in addition to the lack of an understanding of the mechanism by which gabapentin enacarbil (and gabapentin) induces pancreatic acinar cell tumors in rat, makes is difficult to dismiss the human relevance of the rat tumors. For non-genotoxic carcinogens, like gabapentin enacarbil, understanding the mechanism underlying the tumorigenic process is important when assessing the potential for human risk (Cohen SM *et al*. *Toxicol Sci* 78:181-186, 2004; Naito A *et al*. *Comprehensive Toxicology*, 2nd edn. Vol 14, United Kingdom:Elsevier, Ltd., 2010). For example, a body of data was collected for certain peroxisome proliferators (PPAR) that suggested that the formation of pancreatic acinar carcinoma was due to drug-induced decreases in bile acid synthesis or composition, resulting in increases in CCK secretion and pancreatic acinar cell proliferation; increases in CCK are not thought to occur at clinically relevant doses in human. Therefore, for these compounds, mechanistic data allowed a reassessment of human risk. The sponsor conducted only one mechanistic (CCK) study, and was not able to demonstrate a mechanism for gabapentin-induced pancreatic acinar cell tumors in the rat.

Regarding the issue of safety margin, it is my opinion that the data provided by the sponsor do support increasing the safety margin for the pancreatic acinar cell tumors. In the carcinogenicity study of gabapentin enacarbil in rat, the mid dose (2000 mg/kg/day) is arguably a minimum effect dose for pancreatic tumors; however, the low dose (the only dose not associated with pancreatic tumors) was substantially lower (500 mg/kg/day) which resulted in an 8-fold safety margin. In the 2-year carcinogenicity study of gabapentin (Neurontin, the RLD) conducted in rat, the mid dose (1000 mg/kg/day) was

determined to be a "no-effect" dose. The sponsor provided TK bridging data for gabapentin at that dose and the HD (2000 mg/kg/day) used in the gabapentin study. The sponsor's data were similar to those reported by the Neurontin sponsor. Therefore, the sponsor's TK data for gabapentin at 1000 mg/kg/day support an increase in the safety margin from 8 to ≈25. Although there is no clear guidance on an acceptable safety margin for carcinogenicity, a 25-fold margin between plasma exposure (AUC) at the HD used in a lifetime carcinogenicity study and that in humans at the maximum recommended daily dose is an acceptable justification for HD selection; this suggests that tumors observed in animals at plasma exposures beyond 25-fold may be of lesser concern for humans. The sponsor's argument that even higher safety margins exist based on pancreatic tissue levels of gabapentin was not compelling, based on reasons already discussed.

Therefore, from a pharmacology/toxicology standpoint, there is no objection to approval of the NDA, with appropriate labeling.

- **Labeling**

The following labeling recommendations use the sponsor's proposed labeling submitted on October 6, 2010 as a base, and take into account recommendations for the Pregnancy section provided by Dr. Ed Fisher (Pharmacologist, DNP) and additional revisions suggested by the sponsor. Also included, in the Pregnancy and Pediatric sections, is a brief description of published studies reporting adverse effects of gabapentin on development (synaptogenesis) mediated by interaction with the $\alpha_2\delta$-1 subunit of the voltage-activated calcium channel (Eroglu C *et al*. *Cell* 139:380-392, 2009).

Wording from Neurontin labeling (approved 3/1/2011) is included, as appropriate.

HIGHLIGHTS OF PRESCRIBING INFORMATION

----------INDICATIONS AND USAGE----------
[No pharmacologic class is stated, since there is no clear mechanism of action for gabapentin enacarbil's efficacy in RLS.]

----------USE IN SPECIFIC POPULATIONS----------
- Pregnancy: based on animal data, may cause fetal harm. (8.1)

FULL PRESCRIBING INFORMATION

5 WARNINGS AND PRECAUTIONS
5.6 Tumorigenic Potential
In an oral carcinogenicity study, gabapentin enacarbil increased the incidence of pancreatic acinar cell adenoma and carcinoma in male and female rats *[see Nonclinical Toxicology (13.1)]*. The clinical significance of this finding is unknown.

[Additional clinical wording to be taken from the Neurontin label.]

8 USE IN SPECIFIC POPULATIONS

8.1 Pregnancy

Pregnancy Category C. There are no adequate and well-controlled studies with HORIZANT in pregnant women. In nonclinical studies in rat and rabbits, administration of gabapentin enacarbil was developmentally toxic when administered to pregnant animals at doses and gabapentin exposures greater than those used clinically. HORIZANT should be used during pregnancy only if the potential benefit justifies the potential risk to the fetus.

When pregnant rats were administered gabapentin enacarbil (oral doses of 200, 1,000, or 5,000 mg/kg/day) throughout the period of organogenesis, embryo-fetal mortality was increased at the two highest doses and fetal body weights were decreased at the high dose. The no-effect dose for embryo-fetal developmental toxicity in rats is approximately 3 times the recommended human dose (RHD) of 600 mg/day on a body surface area (mg/m^2) basis.

When pregnant rabbits were administered gabapentin enacarbil (oral doses of 200, 500, or 2,500 mg/kg/day) throughout the period of organogenesis, embryo-fetal mortality was increased and fetal body weights were decreased at the high dose. The no-effect dose for embryo-fetal developmental toxicity in rabbits (500 mg/kg/day) is approximately 16 times the RHD on a mg/m^2 basis.

When female rats were administered gabapentin enacarbil (oral doses of 200, 1,000, or 5,000 mg/kg/day throughout the pregnancy and lactation periods, offspring growth and survival were decreased at the two highest doses. The no-effect dose for pre- and post-natal developmental toxicity in rats is approximately 3 times the RHD on a mg/m^2 basis.

In reproductive and developmental studies of gabapentin, developmental toxicity was observed at all doses tested. Increased incidences of hydroureter and/or hydronephrosis were observed in rat offspring following treatment of pregnant animals in studies of fertility and general reproductive performance, embryo-fetal development, and peri- and post-natal development. Overall, a no-effect dose was not established. In mice, treatment of pregnant animals with gabapentin during the period of organogenesis resulted in delayed fetal skeletal ossification at all but the lowest dose tested. When pregnant rabbits were treated with gabapentin during the period of organogenesis, an increase in embryo-fetal mortality was observed at all doses of gabapentin tested.

In a published study, gabapentin (400 mg/kg/day) was administered by intraperitoneal injection to neonatal mice during the first postnatal week, a period of synaptogenesis in rodent (corresponding to the last trimester of pregnancy in human). Gabapentin caused a marked decrease in neuronal synapse formation in brains of intact mice and abnormal neuronal synapse formation in injured mice. Gabapentin has been shown *in vitro* to interfere with activity of the $\alpha 2\delta$ subunit of voltage-activated calcium channels, a receptor involved in neuronal synaptogenesis. The clinical significance of these findings is unknown.

8.2 Labor and Delivery

The effect of HORIZANT on labor and delivery is unknown.

8.3 Nursing Mothers

It is not known whether gabapentin derived from HORIZANT is secreted in human milk; however, gabapentin is secreted into human milk following oral administration of gabapentin products. Because of the potential for serious adverse reactions in nursing infants from HORIZANT, a decision should be made whether to discontinue nursing or to discontinue the drug, taking into account the importance of the drug to the mother.

8.4 Pediatric Use

Safety and effectiveness of HORIZANT in pediatric patients have not been studied.

12 CLINICAL PHARMACOLOGY

12.1 Mechanism of Action

Gabapentin enacarbil is a prodrug of gabapentin and, accordingly, its therapeutic effects in RLS are attributable to gabapentin.

The precise mechanism by which gabapentin is efficacious in RLS is unknown. Gabapentin is structurally related to the neurotransmitter gamma-aminobutyric acid (GABA) but has no effect on GABA binding, uptake, or degradation. Gabapentin enacarbil and gabapentin have been tested in radioligand binding assays, and neither exhibited affinity for a number of other common receptor, ion channel, or transporter proteins.

In vitro studies have shown that gabapentin binds with high affinity to the $\alpha 2\delta$ subunit of voltage-activated calcium channels; however, the relationship of this binding to the therapeutic effects of gabapentin enacarbil in RLS is unknown.

13 NONCLINICAL TOXICOLOGY

13.1 Carcinogenesis, Mutagenesis, Impairment of Fertility

Carcinogenesis: Oral (gavage) carcinogenicity studies were conducted in mice and rats. In mice, gabapentin enacarbil was tested at doses of 500, 2,000, or 5,000 mg/kg/day for up to 104 weeks. There was no evidence of drug-related carcinogenicity. The highest dose tested is 40 times the recommended human dose (RHD) of 600 mg/day, on a body surface area (mg/m^2) basis.

In rats, gabapentin enacarbil was tested at doses of 500, 2,000, or 5,000 mg/kg/day for up to 97 weeks in mid-dose males, 90 weeks in high-dose males, and 104 weeks in females. The plasma exposures (AUC) for gabapentin at these doses are approximately 10, 38, and 75 times, respectively, that in humans at the RHD. Increases in the incidence of pancreatic acinar adenoma and carcinoma were found in mid-dose males and high-dose males and females.

In 2-year dietary carcinogenicity studies of gabapentin, no evidence of drug-related carcinogenicity was observed in mice treated at doses up to 2,000 mg/kg/day. In rats, increases in the incidence of pancreatic acinar cell adenoma and carcinoma were found in male rats receiving the highest dose (2,000 mg/kg), but not at doses of 250 or 1,000 mg/kg/day. At 1,000 mg/kg/day, the plasma AUC for gabapentin is estimated to be approximately 25 times that in humans at the RHD.

Studies designed to investigate the mechanism of gabapentin-induced pancreatic carcinogenesis in rats indicate that gabapentin stimulates DNA synthesis in rat pancreatic

acinar cells *in vitro* and, thus, may be acting as a tumor promoter by enhancing mitogenic activity. It is not known whether gabapentin has the ability to increase cell proliferation in other cell types or in other species, including human.

Mutagenesis: Gabapentin enacarbil was negative in *in vitro* bacterial reverse mutation (Ames) and *in vivo* rat micronucleus assays. In an *in vitro* human lymphocyte assay, there was an increase in the number of chromosomal aberrations with gabapentin enacarbil. This *in vitro* response was attributed to acetaldehyde released by hydrolysis of gabapentin enacarbil during the (b) (4). Acetaldehyde is known to cause chromosome aberrations *in vitro*, but is readily metabolized *in vivo*. The small quantity of acetaldehyde formed from gabapentin enacarbil *in vivo* is rapidly cleared by normal metabolic activity.

Impairment of Fertility: Oral administration of gabapentin enacarbil (doses of 0, 200, 1,000, or 5,000 mg/kg/day) to male and female rats prior to and throughout mating and continuing in females up to day 7 of gestation resulted in no adverse effects on fertility. The highest dose tested is approximately 80 times the RHD on a mg/m^2 basis.

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/s/

LOIS M FREED
04/05/2011

DEPARTMENT OF HEALTH AND HUMAN SERVICES
PUBLIC HEALTH SERVICE
FOOD AND DRUG ADMINISTRATION
CENTER FOR DRUG EVALUATION AND RESEARCH

PHARMACOLOGY/TOXICOLOGY NDA/BLA REVIEW AND EVALUATION

Application number:	NDA 22399
Supporting document/s:	eCTD Sequence Number 0045
Applicant's letter date:	The NDA was resubmitted on January 9, 2009 (Amendment 0004), and filed (Agency letter, 3/13/09)
CDER stamp date:	10/06/2010
Product:	gabapentin enacarbil (HORIZANT)
Indication:	Restless Legs Syndrome
Applicant:	Glaxo Group Limited d/b/a GlaxoSmithKline
Review Division:	Division of Neurology Products
Reviewer:	LuAnn McKinney, DVM, DACVP
Supervisor/Team Leader:	Lois M. Freed, Ph.D.
Division Director:	Russell Katz, M.D.
Project Manager:	Beverly Connor

Disclaimer

Except as specifically identified, all data and information discussed below and necessary for approval of NDA 22399 are owned by GlaxoSmithKline or are data for which GlaxoSmithKline has obtained a written right of reference.
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TABLE OF CONTENTS

1 Executive Summary

1.1 Introduction

NDA 22-399 for XP13512 (HORIZANT ER tablets) , originally submitted on September 15, 2008, withdrawn Nov 11 2008, was resubmitted January 9, 2010. A Complete Response letter was sent on February 17, 2010 and the Complete Response submitted on October 6, 2010. Gabapentin enacarbil is a prodrug, converted to gabapentin during absorption from the GI tract. Gabapentin is approved for treatment of epilepsy and post-herpetic neuralgia; the sponsor seeks approval of gabapentin enacarbil for moderate-to-severe Restless Legs Syndrome (RLS).

1.2 Brief Discussion of Nonclinical Findings

Gabapentin enacarbil is associated with proliferative pancreatic acinar cell changes in male and female Wistar rats (b) (4) 1032-048, Feb 2009). Of particular concern are findings of pancreatic acinar cell adenoma and carcinoma, over concurrent controls, in mid-dose male and high dose male and female rats exposed to gabapentin enacarbil by oral gavage in a 104-week carcinogenicity study. The tumor-free low-dose does not offer an adequate safety margin.

The sponsor has reviewed the original carcinogenicity study, submitted data from *in vivo* and *in vitro* studies of gabapentin enacarbil in rats, mice, and human tissues, and presented written expert opinions.

1.3 Recommendations

1.3.1 Approvability: If the clinical condition warrants and sufficient benefit is derived, gabapentin enacarbil should be approved at a per-patient dose not to exceed 600 mg/day.

1.3.2 Additional Non Clinical Recommendations N/A

2 Drug Information

2.1 Drug

CAS Registry Number: (Optional) 478296-72-9

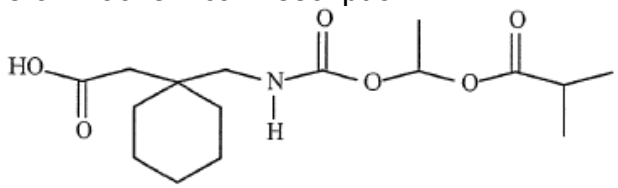
Generic Name: Gabapentin enacarbil

Code Name: XP131512 (XenoPort, Inc. Identification) and GSK1838262 (GSK ID)

Chemical Name: (±-1-([(α-isobutanoyloxyethoxy)carbonyl]-aminomethyl)-1-cyclohexane acetic acid

Molecular Formula/Molecular Weight: C16H27NO6/ 329.40 g/mol

Structure or Biochemical Description:



2.2 Relevant IND: IND 71352 (b) (4) ;

2.3 Drug Formulation : See Non-Clinical Review. 02/01/2010. Source

2.4 Comments on Novel Excipients : See Non-Clinical Review. 02/01/2010**.**

2.5 Comments on Impurities/Degradants of Concern: See Non-Clinical Review. 02/01/2010.

2.6 Proposed Clinical Population and Dosing Regimen: Moderate-to-severe Restless Legs Syndrome. The proposed maximum dose is 600 mg/day.

2.7 Regulatory Background

Gabapentin enacarbil is considered a new chemical entity, and a complete series of nonclinical studies (INDs 71352 (b) (4)) were reviewed by Terry S Peters, DVM. Dr Peters noted a signal for pancreatic tumors in mid and high dose rats in the lifetime carcinogenicity study. Following a review of the data by the ExeCAC, it was concluded that "There was an increased incidence of pancreatic acinar cell hyperplasia, adenomas, and adenomas + carcinomas in males and females at the HD and in MDM" (cf. *Executive CAC meeting minute*s, 8/5/09). The tumor signal is of sufficient concern that a Complete Response action was filed on February 17, 2010. In the Complete Response letter, the narrow safety margin (8-fold for the plasma levels of gabapentin in the NOAEL male rat) was considered too low to justify approval of the 600 mg dose for patients with RLS.

At a subsequent meeting, the sponsor was asked to show that the rat pancreatic acinar tumors are not relevant to human health and it was suggested that the sponsor provide mechanistic data and to rule out a cholecystokinin-mediated effect.

In a Complete Response, October 6, 2010, the sponsor filed a Class 2 Resubmission as a 505 (b)(2). To address the relevance to human of the pancreatic acinar cell tumors detected in rat, and in order to establish that there is an adequate margin of safety, the sponsor submitted their re-review of the original carcinogenicity study, data from new *in vivo* and *in vitro* studies of gabapentin enacarbil in rats, mice, and human tissues, (Appendix A), and presented written expert opinions. (Appendix B)

Studies Submitted

3.1 Studies Reviewed:

2010N 105963: Pilot study of CCK plasma levels in male Wistar rats after a single dose of gabapentin enacarbil
2010N 105807: Toxicokinetic study of gabapentin in male Wistar rats given gabapentin in feed for 14 days: Collection of blood. Determination of Gabapentin concentration in rat whole blood (K2EDTA) Supernatant by LC-MS method.
2010N 105468: Gabapentin PK Pilot study.
2010N 105806: Toxicokinetic study of gabapentin in male Wistar rats given gabapentin in feed for 14 days.
2010N 105808 Toxicokinetic study of gabapentin in male Wistar rats given gabapentin in feed for 14 days. Collection of blood samples.

3.2 Studies Not Reviewed: NA

3.3 Previous Reviews Referenced

Terry S. Peters, DVM- Pharmacology/Toxicology Review and Evaluation, February 1, 2010
Lois M. Freed, Ph.D. Supervisory Pharmacologist, February 5, 2010
Paul C. Brown, Ph.D. Tertiary Pharmacology Review, February 12. 2010
Russell Katz, M.D. Director, Division of Neurology Products, February 15, 2010

4 Pharmacology

4.1 Primary Pharmacology

The pharmacology of gabapentin enacarbil has been thoroughly reviewed by Terry S Peters, DVM. See Non-Clinical Review. 02/01/2010.

Gabapentin enacarbil (HORIZANT) is a pro-drug of gabapentin and is hydrolyzed to gabapentin, isobutyric acid, acetaldehyde, and CO2 during absorption in the GI tract.

Gabapentin enacarbil has no detectable pharmacologic activity until it has been hydrolyzed to gabapentin. Any effects resulting from exposure to gabapentin enacarbil are considered to be attributable to gabapentin, which is well characterized in the published literature.

Previous *in vivo* exposure of rats to ^{14}C-labeled gabapentin and gabapentin enacarbil (PK –R-450-13512, September, 2003) demonstrated that pancreas in the male rat contained the highest levels of radioactivity 2 hours post-dose. Excretion of gabapentin enacarbil is primarily renal and by 6 hr post-dose, only kidneys (both genders), pancreas (males only), pituitary (males only) and carcass (males only) retained amounts of radioactivity greater than 10 µg equiv/g. Essentially no radiolabel was present at 24 hrs post-dosing. The majority of the radiolabel was recovered in urine, primarily in the first 12 hrs.

5.2 Toxicokinetics: Day 14, blood levels of repeat oral daily doses of XP131512 (Gabapentin enacarbil) and Gabapentin

Dose	Sex	C_{max} Gabapentin Enacarbil	C_{max} Gabapentin	T_{max} Gabapentin Enacarbil	T_{max} (hr) Gabapentin	AUC Gabapentin Enacarbil	AUC Gabapentin
500	M	139		0.5		457	
500	F	120		0.5		418	
1000	M		59.8		9.0		1090
1000	F	---	---	---	---	---	---
2000	M	204	79.7	4.0	9.0	2060	1500
2000	F	175	---	0.5		1610	
5000	M	375		4.0		4840	
5000	F	340		4.0		3630	

General Toxicology, Carcinogenicity, Pharmacology:

See Pharmacology/Toxicology Review and Evaluation, Dr. Terry S. Peters, February 1, 2010

See Memorandum: Lois M. Freed, Ph.D. Supervisory Pharmacologist, February 5, 2010

Integrated Summary and Safety Evaluation

Gabapentin, although neither mutagenic nor clastogenic, is a weak mitogen and is positively associated, by an unknown mechanism, with an increased incidence of pancreatic acinar cell adenoma and carcinoma in male Wistar rats. (Radulovic LL *et al Drugs Today* 31(8):P597-611,1995). In the published literature, gabapentin-dosed HDM had single tumors per pancreas, except in two animals that had two adenomas, and

female rats were free of tumors. The reported pancreatic acinar cell tumors were "…considered low grade since they did not invade adjacent tissues, metastasize or cause the death of any animal…" (Radulovic LL et al., 1995).

In the gabapentin enacarbil study in Wistar rats, adenoma (greater than controls) and carcinoma were seen in a HD male and in a HD female. Adenoma and one carcinoma were seen in MD males. The carcinoma were found to be locally invasive (although no metastases were found) and carcinoma was the cause of death in the MD male.

104 week oral gavage by gabapentin enacarbil in Wistar Rats.
Pancreatic tumor incidence as %.

	Males (N=60/dose group)				Females (N=60/dose group)			
	0 mg/kg	500 mg/kg	2000 mg/kg	5000 Mg/kg	0 mg/kg	500 mg/kg	2000 mg/kg	5000 Mg/kg
Adenoma	3.3%	6.7%	6.7%	13.3%	0	0	0	5%
Carcinoma	0	0	1.7%	1.7%	0	0	0	1.7%
Total Benign+ Malignant	3.3%	6.7%	8.3%	15%	0	0	0	6.7%

In her Memorandum, Dr. Freed provides the following table:

FINDING	MALES				FEMALES			
	0	500	2000	5000	0	500	2000	5000
NEOPLASTIC								
adenoma	2/60	4/60	4/60	8/60	0/60	0/60	0/60	3/60
carcinoma	0/60	0/60	1/60	1/60	0/60	0/60	0/60	1/60
total	**2/60**	**4/60**	**5/60**	**9/60**	**0/60**	**0/60**	**0/60**	**4/60**
NON-NEOPLASTIC								
hyperplasia								
minimal	8/60	2/60	4/60	5/60	1/60	0/60	2/60	5/60
mild	3/60	6/60	7/60	12/60	0/60	0/60	1/60	5/60
moderate	3/60	1/60	3/60	3/60	0/60	1/60	1/60	4/60
severe	0/60	1/60	0/60	0/60	0/60	0/60	0/60	0/60
total	**14/60**	**10/60**	**14/60**	**20/60**	**1/60**	**1/60**	**4/60**	**14/60**

Compared to concurrent controls, there is a dose-related increase in pancreatic acinar cell adenoma and carcinoma and an increase in the severity of hyperplasia in MD male and HD male and female rats. Carcinoma was described in the study pathologist's report as "locally invasive without evidence of distant metastases"; the acinar cell carcinoma was the cause of death in the affected MD male, but not in the HD male and female rats. Because of morbidity due to chronic nephropathy (gabapentin is excreted through the kidney), the HD males were terminated at 90 weeks and the MD males at

97 weeks. The study was of deemed to be of sufficient length to have accurately detected proliferative changes.

Pancreatic proliferative changes are rare in male and female Wistar-Han rats and in female Wistar rats and uncommon in male Wistar rats. Because pancreatic acinar tumors are rare in female Wistar rats and, in addition to the carcinoma in HDM and HDF, there is a clear increase in adenoma in both sexes; adenoma and carcinoma in males and females at 5000 mg/kg (HD) are clearly a drug effect.

At the time of the original study, the contract pathologist's in-house historic database combined data from Wistar and Wistar-Han control rats. When assessed for Wistar rats alone, the database is too small for statistical comparisons, and no carcinoma were found. Based on larger, published Wistar rat tumor incidence tables from 1994 and 1998, at least one spontaneous pancreatic acinar carcinoma would have been found within the 240 male rats in the four dose groups. The sponsor concludes that the carcinoma in a MDM is a spontaneous lesion, within the anticipated range for the strain, and that the MD of 2000 mg/kg is the NOEL for gabapentin-induced carcinoma.

The same incidence tables are used to argue that adenoma at MD and LD are within anticipated incidence rates and that the incidence in the concurrent controls is unusually low. The adenoma incidence in LD and MD males are within the published and in-house ranges and the sponsor contends that there is no drug-related increase of adenoma in either the LD or MD males.

Upon review of the concurrent and historic controls, the sponsor concludes the 6.6% incidence of adenoma in LD and MD males and the carcinoma in one MDM is spontaneous, rather than a drug effect. The in-house database used to support that interpretation of concurrent controls is too small to offer statistical inference but, although no carcinoma were seen, does show an incidence of 6.5% adenoma,.

Pancreatic acinar cell hyperplasia is historically of variable severity and the incidence at the MD was the same as or less than concurrent controls. The incidence in the HD males and females is agreed to be due to gabapentin. The sponsor finds the interpretation of slight increased severity of the MD proliferative lesions to be "conservative and equivocal and … largely based on consideration of the published data with gabapentin, in particular the toxicokinetic data for gabapentin, which has been published only in a figure and not in tabular form."

The sponsor concludes that absent an increase in either hyperplasia or adenoma (above in-house data and published incidence tables), the carcinoma in one MDM is spontaneous, and "the NOEL for carcinoma is close to 2000 mg/kg/day", "close to 38-fold" margin of safety.

Mechanistic studies:

In a review of the literature, of previously submitted studies, and of the i*n vivo* and *in vitro* studies submitted with the Complete Response (see Appendices A and B), the sponsor argues that the rat pancreas is uniquely exposed to high levels of gabapentin after oral dosing by gabapentin or gabapentin enacarbil.

The rat pancreas does have uniquely high levels of CCK (cholecystokinin) receptors, and diet-induced CCK is known to stimulate the growth of normal pancreas in rats and to selectively stimulate the growth of carcinogen-induced hyperplastic acinar cell lesions. These proliferative effects are considered to be species-specific to the rat and are accepted to not have implications for human health. In a pilot study (2010N 105963), rats were administered oral gavage of 5000 mg/kg gabapentin in vehicle, 4000 mg/kg casein in vehicle, or 10 mL/kg corn oil. In measurements out to two hours, gabapentin did not cause CCK elevations detectably different from vehicle and, although more than corn oil, substantially less than the positive control (casein). Any drug-associated pancreatic acinar proliferative changes are thus attributed to some mechanism other than the cholecystokinin mechanism (de la Iglesia FA, et al, *Toxicologist*, 1997;36:178).

Previous *in vivo* exposure of rats to ^{14}C-labeled gabapentin and gabapentin enacarbil (PK –R-450-13512, September, 2003) demonstrated that in the male rat, pancreas contained the highest levels of radioactivity 2 hours post-dose. At 6 hr post-dose, kidneys (both genders), pancreas (males only), pituitary (males only) and carcass (males only) retained amounts of radioactivity greater than 10 µg equiv/g. From the published literature, the sponsor concluded that mice had a similar tissue distribution of gabapentin but lower blood concentrations, and thus lower absolute tissue levels than the rat. Also from published literature, the pancreas-to-blood ratio in gabapentin-dosed primates did not exceed 1:1, indicating no concentration of gabapentin in pancreas.

In experiment 2010N105598 (August, 2010), sections of human and rat pancreas were exposed *in vitro* to tritiated gabapentin. The human tissues were harvested 17-21 hours before sectioning, and the rat tissues harvested on site that day. Exposure was at 37° C in a perfusion chamber used in publications of similar studies. (Balkenohl et al., Epilepsia, 1993; 34: 157). After 3 hours, rat pancreas tissue slices contained 11-fold greater gabapentin-associated radioactivity than human pancreas tissue slices. This argues that there is a substantial inter-species difference in accumulation of gabapentin in human vs rat pancreas.

Gabapentin is a zwitterion and trans-membrane transport requires active transporter proteins. Study XD2010 was performed to see if there is a species difference in pancreatic gabapentin transporter protein levels. In XD2010, gabapentin carrier proteins were determined by injecting cRNA from plasmid-transfected cell lines into *Xenopus laevis* oocytes and recording intracellular uptake of gabapentin. The presence of those gabapentin carrier proteins was determined using real time quantitative PCR on total RNA isolated from pancreas of human, Wistar rat, Sprague-Dawley rat and B6C3F1 mouse. By fluorescent immunohistochemistry, the proteins in rodents were found distributed uniquely in pancreatic exocrine acinar cells. In contrast, the distribution

in human pancreas was uniquely in endocrine Islet cells. The sponsor contends that the total mass of acinar cells in pancreas explains the higher concentration of gabapentin in the rat pancreas.

The sponsor then calculated that at the 500 mg/kg dose (~8 fold safety margin by AUC), the 11-fold concentration in rat pancreas, attributed to the transport protein expression in rat acinar cells, would "*provide a safety margin of >50-fold, based on at least a 5-fold higher tissue accumulation ratio in rats"* and further that " *Exposure levels of the active drug in the target organ are the most relevant parameter."*

Expert opinions on the relevance of pancreatic acinar carcinoma to human health
Three experts in rat and pancreatic carcinogenesis offered written opinions on the relevance of the tumors to human health.

(b) (4), MD concluded that there are "multiple reasons to doubt the relevance of the acinar cell adenomas and carcinomas seen the toxicologic studies of gabapentin enacarbil in Wistar rats for human risk assessment.**"** In a discussion of pancreatic intraepithelial neoplasia (antecedent to ductal carcinoma) and acinar-to-ductal metaplasia (ADM), he concluded "in the absence of K*ras* mutation, acinar cells do not give rise to ductal adenocarcinomas that is similar to the dominant pancreatic cancer in humans."

(b) (4), M.D., Ph.D. addressed the comparison of the original gabapentin carcinogenicity studies and the more recent gabapentin enacarbil carcinogenicity studies. Dr. (b) (4) pointed out that "Because of evolving differences in the strains over time [and] differences in Purina certified diets, systemic exposure at the different doses cannot necessarily be used to compare … the different Gabapentin and Gabapentin enacarbil carcinogenicity studies." And he concludes that "The rat model itself with regard to pancreatic acinar cell carcinogenesis, is not relevant to the human situation. It is predicted with considerable confidence that no carcinogenic effect will occur in humans."

(b) (4), MD concludes that " based on the finding of only pancreatic acinar cell neoplasms in the Gabapentin enacarbil (GEn) rat bioassay, a likely epigenetic mode of action, and the dose response relationships for this neoplasm relative to human exposures, I conclude that GEn at the therapeutic dose does not represent a cancer risk to patients.

The sponsor concludes that*" Based on the weight of the evidence, the high safety margin for carcinomas and the lack of relevance of rat acinar cell tumors to human cancer indicate an insignificant cancer risk to humans from the proposed human dose of 600 mg gabapentin enacarbil for moderate-to-severe RLS."*

Reviewer Summary:

Worldwide, pancreatic cancer ranks 13th in incidence but 8th as a cause of cancer death (Anderson KE, In: *Cancer Epidemiology and Prevention*. 3rd Ed. 2006). The most common form of human pancreatic carcinoma has a ductal morphology, is characterized by *Kras* mutations, and has long been thought to arise from pancreatic ducts or ductal stem cells. Recent reports also implicate acinar cells and islet cells as a possible cell of origin. (Pour PM et al, Mol Cancer, 2: 13, 2003) Pancreatic acinar cell carcinoma is a rare tumor in humans, and all three experts argue convincingly that rat tumors of this cell type are unlikely to be predictive of pancreatic ductal tumors in humans.

The rat susceptibility to pancreatic acinar tumors is sex and strain-related and these tumors do occur spontaneously, albeit uncommonly, in male Wistar rats. Compound-induced increases in rare or uncommon spontaneous lesions are difficult to interpret, and historical control databases are a comparison tool that can aid interpretation if it is of sufficient size, well defined, with consistent diagnostic nomenclature, and taken from studies finalized not more than 7 years previous. (Keenan et al., *Toxicol Pathol,* vol. 37(5): pp. 679-693, 2009).

Dr (b) (4) points out that changes in strain, husbandry, and diagnostic criteria over time can preclude comparison between studies separated by many years. The published incidence tables the sponsor uses to argue the spontaneity of the MDM carcinoma are not less than 13 years old; this is beyond the two-to-seven years recommended by the Society of Toxicologic Pathology (Keenan, et al, 2009). The tables are compiled from studies of undefined dietary regimen, and the authors note that the incidences in the 1998 database are skewed by a 50% incidence of adenoma and 17% incidence of carcinoma in one particular study. Excluding that study, both publications find an incidence of ~2.5% for adenoma and ~.5% for carcinoma in male Wistar rats. (The incidence ranges from 0-10% and 0-2% respectively.) The 1998 incidence tables are used to argue that the adenoma incidence in the concurrent controls (3.3%) is unusually low. The in-house database used to support that interpretation of the concurrent controls is too small to offer statistical comparisons, but the incidence of adenoma is comparatively high (6.5%).

The combined Wistar-Han+Wistar rat historical control data that aided the initial interpretation of the carcinogenicity study is inappropriate, and the Wistar-only in-house control data are too few to predict spontaneous lesions and are not well characterized. The sponsor argues that the carcinoma in the MD male is an anticipated spontaneous occurrence and that that dose level should be considered the NOEL for tumor. Certainly the carcinoma signal at 2000 mg/kg is low (one out of 40 male rats), but the tumor is locally invasive and was determined to be the cause of death in the rat. The rats were euthanized at 97 out of 104 days, and other tumors, or more clearly diagnosed hyperplasia, may have been seen at a later time point.

Concurrent controls remain the single best comparator (Keenan, et al, 2009) and because of the increase over concurrent controls of carcinoma and adenoma at the mid dose, the clear no-tumor level is the low-dose of 500 mg/kg. This exposure level does

not offer sufficient safety margins for human doses at 600 mg/day for treatment of Restless Legs Syndrome.

The findings of the distribution of gabapentin-carrier proteins are striking. That there are many more cells able to transport gabapentin across the cell membrane in rat pancreas than in human is undeniable. There is a smaller, separate, population of islet cells in the human that expresses similar protein levels. While there are no data to demonstrate that gabapentin is preferentially taken up by islet cells, the concentrations of the transporter proteins would indicate that this population could be exposed to levels of gabapentin similar to that of acinar cells in rodents. As stated in the meeting minutes (5/18/2010), "*Published data suggest that gabapentin may be a mitogen; therefore, neoplasms could potentially develop in any tissue exposed to sufficient concentrations of gabapentin."* Further, neuroendocrine islet *Beta*-cells are capable of transdifferentiation to ductal cells, (Pour PM et al, 2003; Jopling C, et al, Nature Reviews, Molecular Cell Biology, 12:70-89, 2011) and exposure to a mitogen, at levels that the carrier proteins might allow, is reason for continued concern.

Conclusion:

The sponsor has provided convincing evidence that rat acinar cells have an abundance of gabapentin carrier proteins (*in vitro*) and that the rat pancreas does take up high levels of gabapentin post-oral-dose *in vivo*.

The sponsor presents adequate evidence that acinar cell tumors are rare in humans and that the rat acinar cell tumors do not express the genetic, morphologic or antecedent changes of the more common pancreatic ductal adenocarcinoma in humans.

Based on the tissue-slice uptake differences between rat and human pancreas, the sponsor states that "*(500 mg/kg/day), must be considered to provide a safety margin of >50-fold, based on at least a 5-fold higher tissue accumulation ratio in rats."* Speculatively, the same rationale might well apply to the neuroendocrine cells of the human pancreas. There are no data to support or discount either possibility, and neither conclusion is accepted.

In comparison to concurrent controls, the data establish a clear carcinogenic dose at 5000 mg/kg, show a weak carcinogenic effect at the mid dose of 2000 mg/kg in Wistar rat pancreas and no effect at 500 mg/kg. Comparing to less-than-ideal historic control incidence data, the sponsor poses that the no-effect dose level for tumor would be "close to 2000 mg/kg/day" and that the safety margin would be "close to 38-fold".

The highest no-tumor dose level in the rat is likely somewhere greater than 500 mg/kg but less than the mid-dose of 2000 mg/kg. The margin of safety at the mid-dose would be 38-fold, and at the low dose is 8-fold. It is likely that there could well be a tumor-free dose, providing at least a 25-fold margin of safety, at a level "close to" but less than 2000 mg/kg.

There is a weak, but worrisome, signal for carcinogenicity. The risk is relatively low however and, if the clinical condition warrants and sufficient benefit is derived, the proposed dose of not more than 600 mg/day of gabapentin enacarbil should be approved.

12 Appendix/Attachments

Appendix A.
Review of Nonclinical studies submitted by GlaxoSmithKline in a Complete Response, dated 10/6/ 2010

1. Pilot Study of CCK Plasma Levels in Male Wistar Rats after a Single Dose of Gabapentin Enacarbil

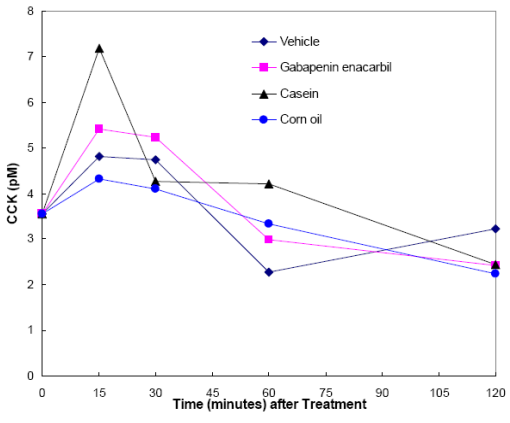
Study no.:	2010N105963
Conducting laboratory and location:	XenoPort, Inc 3413410 Central Expressway Santa Clara, CA 95051-0703
Date of study initiation:	2 Sept 2010
GLP compliance:	No
QA statement:	Yes
Drug, lot #, and % purity:	Gabapentin enacarbil (GEn XP13512), Lot No 43. 100.3% w/w

Key Study Findings:
1) The net increase in CCK in the gabapentin enacarbil treatment group over vehicle control group, as measured by the two laboratories, was 0.6 pM and 2.5 pM, while the CCK concentrations in the vehicle control group were 4.8 pM and 3.1 pM, respectively
2) Pure corn oil appeared to elicit no more of a response than the vehicle control. CCK concentrations in the vehicle control groups were higher than baseline blood samples.

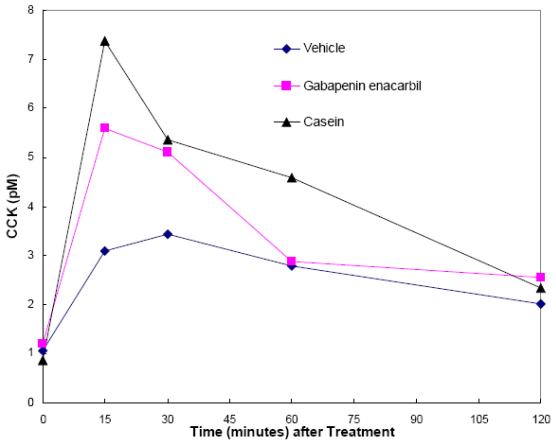
Methods

Doses:	Vehicle, 5000mg/kg gabapentin in vehicle, 4000mg/kg casein in vehicle, corn oil 10mL/kg.
Frequency of dosing:	once
Route of administration:	Oral gavage
Dose volume:	20 mL/kg.
Formulation/Vehicle:	0.5%methylcellulose/0.1%Tween80 in distilled water
Species/Strain:	Male Wistar rats
Number/Sex/Group:	15 males/group
Age:	
Weight:	252-310 grams
Satellite groups:	NA
Unique study design:	Parallel analyses at GSK and (b) (4) Radioimmunoassay kit from Euro-Diagnostica for measurement of CCK. The assay is reported to detect concentrations of CCK-8 as low as 0.3 pM
Deviation from study protocol:	None

(b) (4)



GSK:



The Sponsor concluded that "it is not possible to reproducibly measure subtle change in plasma CK over and above the CCK levels in the vehicle control group."

Comment: The experiments appear adequately controlled, the analyses were performed at separate laboratories. There does not appear to be a CCK mechanism to account for the pancreatic tumors in Wistar rats fed Gabapentin, nor would there be an expected CCK increase in rats fed gabapentin enacarbil.

2. Expression and Localization of Gabapentin Transporter Proteins in Human, Rat and Mouse Pancreatic Tissue

Study no.:	XD2010/00358/00
Conducting laboratory and location:	Xenoport, Inc, Santa Clara, CA
Date of study initiation:	11 May, 2010
GLP compliance:	No
QA statement:	No

1) The purpose of this portion was to identify those proteins that actively transport gabapentin across cell membranes. Gabapentin transporter uptake studies were conducted in either transporter-expressing mammalian (HEK) cells or *Xenopus laevis* oocytes injected with transporter-encoding cRNA .

Based on transfected Oocytes:

LAT1 forms a heterodimer with the heavy chain 4F2hc, which is required for LAT1 trafficking to the cell surface.

Oocytes that expressed LAT1/LAT2 and 4F2hc showed a high level of L-phenylalanine uptake compared to uninjected oocytes, indicating that the transporter is functionally expressed

LAT1/4F2hc mediated gabapentin uptake was saturable and fitted to the Michaelis Menten equation (pEC50 = 3.8 ± 0.3).

LAT2/4F2hc did not recognize gabapentin.

Dose-response curves for LAT3 and LAT4 did not saturate.

2) To identify which of those proteins are present in pancreas from the three species, mRNA expression analysis was performed using real time quantitative PCR on total RNA isolated from pancreas of human, Wistar rat, Sprague-Dawley rat and B6C3F1 mouse. [Frozen pancreas harvested at no more than one hour post-cardiac arrest, from a 76 year old female, a 83 year old Caucasian female and a 44 year old male human. Pancreas from Sprague Dawley and Wistar rats and B6C3F1 mice frozen within 1 hour of resection.]

Subset of transporter genes were expressed at significantly higher levels (>10-fold) in rat pancreas compared to human, and also were expressed at high absolute levels (>10% of GAPDH). This includes LAT1, LAT2, 4F2hc, MCT6, MCT7, PAT1, and SN2.

A high degree of concordance was observed for gene expression levels between Wistar and Sprague-Dawley strains, and similar results were obtained when comparing B6C3F1 mouse transporter gene expression profiles to human.

3) The anatomic location of the transporters in tissue sections was determined by fluorescence immunohistochemistry.

In both human and rat, the localization pattern of 4F2hc closely resembled that of LAT1.

Rodents: The Gabapentin transporter proteins LAT1 and 4F2hc, are overwhelmingly located in acinar cells; none in are in islets.

Human: The Gabapentin transported proteins LAT1 and 4F2hc, are uniquely in islet cells; none in acinar cells.

The staining pattern of LAT2 was virtually identical to that of LAT1 and 4F2hc.

The sponsor concluded: "Acinar cells are far more abundant than islet cells in the pancreas, and taken together this data supports the premise that pancreatic accumulation of gabapentin from the blood is primarily mediated through active uptake by the LAT1/4F2hc and Lat1/4F2hc transporters, with the differential expression of these gene products accounting for the higher concentrations of gabapentin observed in rodent vs. primate pancreatic tissue."

Conclusion: Due to the relative mass of acinar vs. islet cells in the mammalian pancreas, the sponsor presents a reasonable rationale. However, while there are no data to demonstrate that gabapentin is preferentially taken up by islet cells, the concentration of the transporter proteins would indicate that this population would be exposed to levels of gabapentin similar to that of acinar cells in rodents. As stated in the meeting minutes (5/18/2010), "*Published data suggest that gabapentin may be a mitogen; therefore, neoplasms could potentially develop in any tissue exposed to sufficient concentrations of gabapentin.*"

3. In Vitro Study of Accumulation of Gabapentin in Pancreas Slices of Rat and Human

Study no.:	2010N105598
Conducting laboratory and location:	XenoPort, Inc, Santa Clara, CA
Date of study initiation:	26 August, 2010
GLP compliance:	No
QA statement:	No

This study repeats the work of Balkenohl et al. (Balkenohl MW, Turck D, Kirste G and Feuerstein TJ, Species Specific Accumulation of Gabapentin in Male Rat Pancreatic Tissue Compared to Human and Monkey Pancreas Epilepsia, 1993; 34: 157). The tissue perfusion apparatus (superfusion chambers) were provided by (b) (4), a co-author of that paper.

Human pancreas was collected within 1 hour of cessation of cardiovascular circulation, shipped on wet ice and delivered within 17-21 hours of harvest. Rat pancreas was harvested on site on the day of the study.

4-12 mg vibratome-sectioned slices of pancreas from 4 Wistar male rats and 3 individual male human donors (6 slices per pancreas), were perfused at 37 degrees C with perfusion medium for 30 minutes, tritiated gabapentin for 45 minutes and perfusion medium for 30 minutes. Radioactivity was measured at 0 and 3 hours in dpm/mg in the tissue and the perfusion medium.

In this study, rat pancreas showed an approximately 11-fold higher accumulation of gabapentin-associated radioactivity compared to humans.

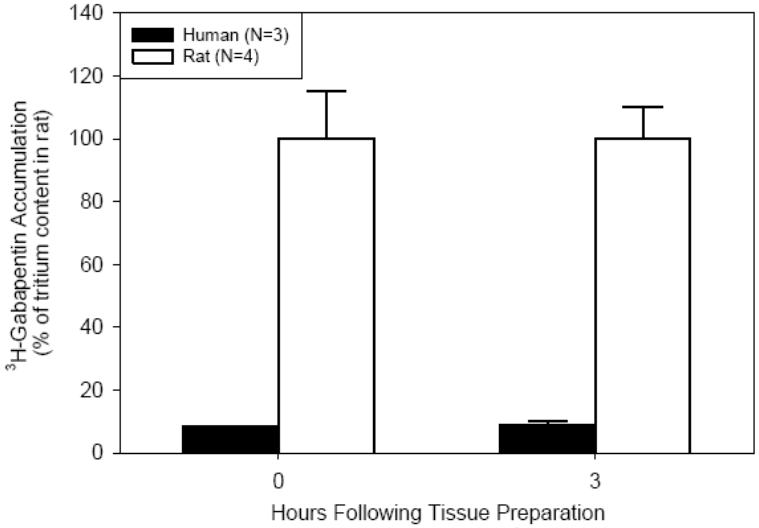


Figure 2.2: Mean (SEM) relative accumulation of ^{3}H-gabapentin radioactivity in rat and human pancreas slices after in vitro perfusion

Table 6.2. Mean ^{3}H Ratio of Human Pancreas Slice/Medium at 0 hour and 3 hours after Tissue Preparation

Human ID	Sample	Time (hr)	Mean dpm/mg	Pancreas/medium ^{3}H Ratio
1	Slice*	0	160	0.156
	Medium		1030	
2	Slice*	0	173	0.161
	Medium		1076	
3	Slice*	0	170	0.153
	Medium		1113	
0 hour data			Mean	0.156
			SEM	0.002
Human ID	Sample	Time (hr)	Mean dpm/mg	Pancreas/medium ^{3}H Ratio
1	Slice*	3	154	0.150
	Medium		1030	
2	Slice*	3	223	0.208
	Medium		1076	
3	Slice#	3	146	0.131
	Medium		1113	
3-hour data			Mean#	0.163
			SEM#	0.023
Combined 0-hour and 3-hour data			Overall Mean#	0.160
			Overall SEM#	0.010

* n = 6.
# n = 5; One slice at time 3 hours for Human 3 was considered an outlier and was excluded from the analysis.

Table 6.1. Mean ^{3}H Ratio of Rat Pancreas Slice/Medium at 0 hour and 3 hours after Tissue Preparation

Rat ID	Sample	Time (hr)	Mean dpm/mg	Pancreas/medium ^{3}H Ratio
1	Slice*	0	1753	1.65
	Medium		1061	
2	Slice*	0	1356	1.33
	Medium		1016	
3	Slice*	0	1862	1.77
	Medium		1050	
4	Slice*	0	2857	2.64
	Medium		1082	
0 hour data			Mean	1.85
			SEM	0.280
Rat ID	Sample	Time (hr)	Mean dpm/mg	Pancreas/medium ^{3}H Ratio
1	Slice*	3	1886	1.78
	Medium		1061	
2	Slice*	3	1763	1.74
	Medium		1016	
3	Slice*	3	1483	1.41
	Medium		1050	
4	Slice*	3	2467	2.28
	Medium		1082	
3 hour data			Mean	1.80
			SEM	0.179
Combined 0 hour and 3 hour data			Overall Mean	1.83
			Overall SEM	0.154

* n = 6.

4. Blood-to-Plasma Ratio of Gabapentin in Mouse Blood

Study no.: 2010N105641

Conducting laboratory and location: XenoPort, Inc
3413410 Central Expressway
Santa Clara, CA 95051-0703

Date of study initiation:

GLP compliance:

QA statement:

Drug, lot #, and % purity: No 288003702, from (b) (4) 100% purity

Key Study Findings:

The mean blood-to-plasma ratios of gabapentin were nearly 1.0 in the blood of B6C3F1 mice regardless of the gender.

Methods: *in vitro* studies designed to examine the blood to-plasma ratio of gabapentin in the blood obtained from male and female B6C3F1 mice.

Doses: Gabapentin at 50, 100, 200 and 300 µg/mL was incubated with blood from both male and female mice for 30 min or for one hour at 37ºC.

Formulation/Vehicle: In PBS at 50 mg/mL

Table 5.1 Blood-to-Plasma Ratio of Gabapentin in the Blood of Male B6C3F1 Mice

Nominal Concentration of Gabapentin in the Blood, Incubation Time	Mean Blood Concentration (µg/mL)	Mean Plasma Concentration (µg/mL)	Blood-to-Plasma Ratio
50 µg/mL, 60 min	61	58	1.1
100 µg/mL, 60 min	108	104	1.0
200 µg/mL, 60 min	182	170	1.1
300 µg/mL, 60 min	268	260	1.0
300 µg/mL, 30 min	268	279	1.0

Table 5.2 Blood-to-Plasma Ratio of Gabapentin in the Blood of Female B6C3F1 Mice

Nominal Concentration of Gabapentin in the Blood, Incubation Time	Mean Blood Concentration (µg/mL)	Mean Plasma Concentration (µg/mL)	Blood-to-Plasma Ratio
50 µg/mL, 60 min	41	48	0.9
100 µg/mL, 60 min	92	105	0.9
200 µg/mL, 60 min	190	198	1.0
300 µg/mL, 60 min	280	284	1.0
50 µg/mL, 30 min	40	44	0.9
200 µg/mL, 30 min	201	197	1.0
300 µg/mL, 30 min	305	301	1.0

Conclusion: gabapentin freely distributes between erythrocytes and plasma in the blood of mice.

5. Pilot Toxicokinetic Study of Gabapentin in Male Wistar Rats Given Gabapentin in Feed for 7 Days

Study no.:	2010N105486/ XP100-TK
Conducting laboratory and location:	XenoPort, Inc 3413410 Central Expressway Santa Clara, CA 95051-0703
Date of study initiation:	August 2010
GLP compliance:	No
QA statement:	No
Drug, lot #, and % purity:	Commercial gabapentin from (b) (4), purity 100.1% w/w.

Key Study Findings
Gabapentin plasma concentrations (µg/mL) = 0.55 + 1.18 × gabapentin blood concentrations (µg/mL).
Steady state exposure to gabapentin in blood and plasma (based on Cmax and AUC) was less than proportional to gabapentin dose at the dose levels of 1000 to 2000 mg/kg/day.

Methods

Doses:	0. 1000. 2000 mg/kg
Frequency of dosing:	Daily
Route of administration:	diet
Formulation/Vehicle:	Certified Rodent Chow 5002
Species/Strain:	Male Wistar Rats
Number/Sex/Group:	7/ group
Weight:	194 to 233 g.

Observations and Results

Gabapentin plasma concentrations (µg/mL) = 0.55 + 1.18 × gabapentin blood concentrations (µg/mL)

Table 2.1: Mean ± SD Toxicokinetic Parameters of Gabapentin in Blood and Plasma on Day 7 Following Repeated Oral Administration of Gabapentin in Diet for 7 Days in Male Wistar Rats

Gabapentin Target Dose (mg/kg/day)	Gabapentin Actual Dose On Day 7 (mg/kg/day)	N	PK Parameters of Gabapentin in Blood			
			No Dose Correction		With Dose Correction to 1000 or 2000 mg/kg/day	
			C_{max} (µg/mL)	AUC_{0-24} (µg*hr/mL)	C_{max} (µg/mL)	AUC_{0-24} (µg*hr/mL)
1000	654 ± 109	7	48.5 ± 5.15	748 ± 81.9	76.1 ± 16.1	1160 ± 150
2000	1810 ± 273	6	84.2 ± 11.0	1490 ± 256	95.1 ± 20.1	1670 ± 340
Gabapentin Target Dose (mg/kg/day)	**Gabapentin Actual Dose On Day 7 (mg/kg/day)**	**N**	**PK Parameters of Gabapentin in Plasma [a]**			
			No Dose Correction		**With Dose Correction to 1000 or 2000 mg/kg/day**	
			C_{max} (µg/mL)	AUC_{0-24} (µg*hr/mL)	C_{max} (µg/mL)	AUC_{0-24} (µg*hr/mL)
1000	654 ± 109	7	58.5 ± 8.19	883 ± 108	91.3 ± 18.0	1370 ± 160
2000	1810 ± 273	6	99.4 ± 12.3	1770 ± 276	112 ± 22.3	1990 ± 405

[a] Gabapentin concentrations in plasma samples prior to the start of the dark cycle were projected from blood data (0 hr) using the following equation: gabapentin plasma concentrations (µg/mL) = 0.55 + 1.18 × gabapentin blood concentrations (µg/mL).

Concentrations of gabapentin in plasma reached a maximum between 2 hr and 12 hr after the start of the dark cycle.

Concentrations of gabapentin in blood reached a maximum between 2 hr and 12 hr after the start of the dark cycle.

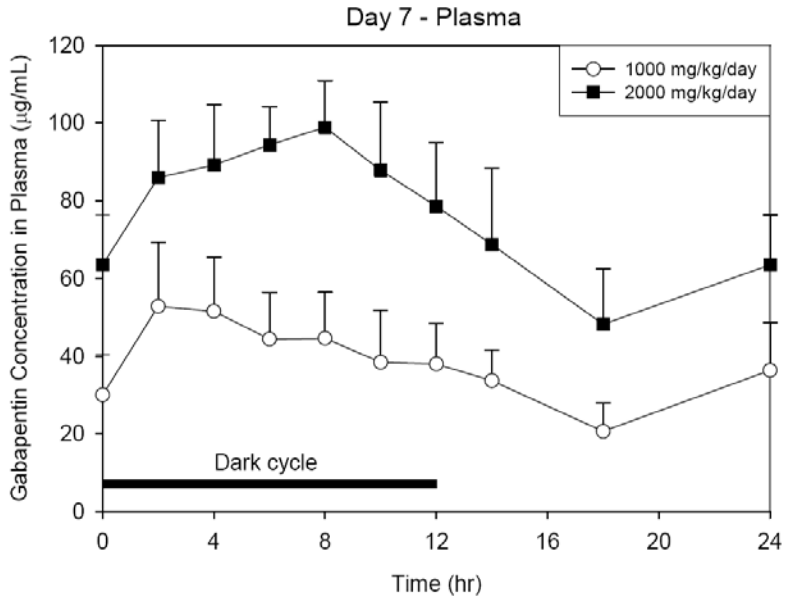


Figure 7.3. Mean (SD) concentration-time profiles of gabapentin in plasma on Day 7 following repeated oral administration of gabapentin in diet for 7 days in male Wistar rats

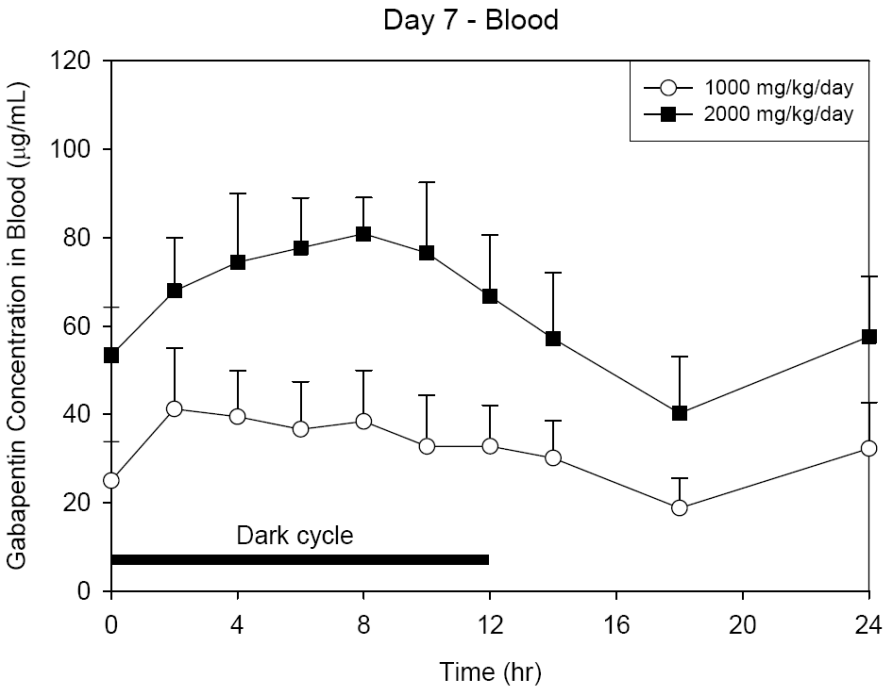


Figure 7.2. Mean (SD) concentration-time profiles of gabapentin in blood on Day 7 following repeated oral administration of gabapentin in diet for 7 days in male Wistar rats

6. Toxicokinetic Study of Gabapentin in Male Wistar Rats Given Gabapentin in Feed for 14 Days. And collection of blood samples.

Study no.: 2010N105806, 2010N105808_00
Conducting laboratory and location: (b) (4)
Date of study report: August 31 2010
GLP compliance: yes
QA statement: yes
Drug, lot #, and % purity: (b) (4) Purity 100.2%.

Key Study Findings:

1) The Cmax values in blood increased in the ratio of 1:1.3 for the dose increase ratio of 1:2,
2) The AUC0-24 values in blood increased in the ratio of 1:1.4 for the dose ratio of 1:2.
3) Steady state exposure to gabapentin in blood was less than proportional to gabapentin
dose at the dose levels of 1000 to 2000 mg/kg/day.

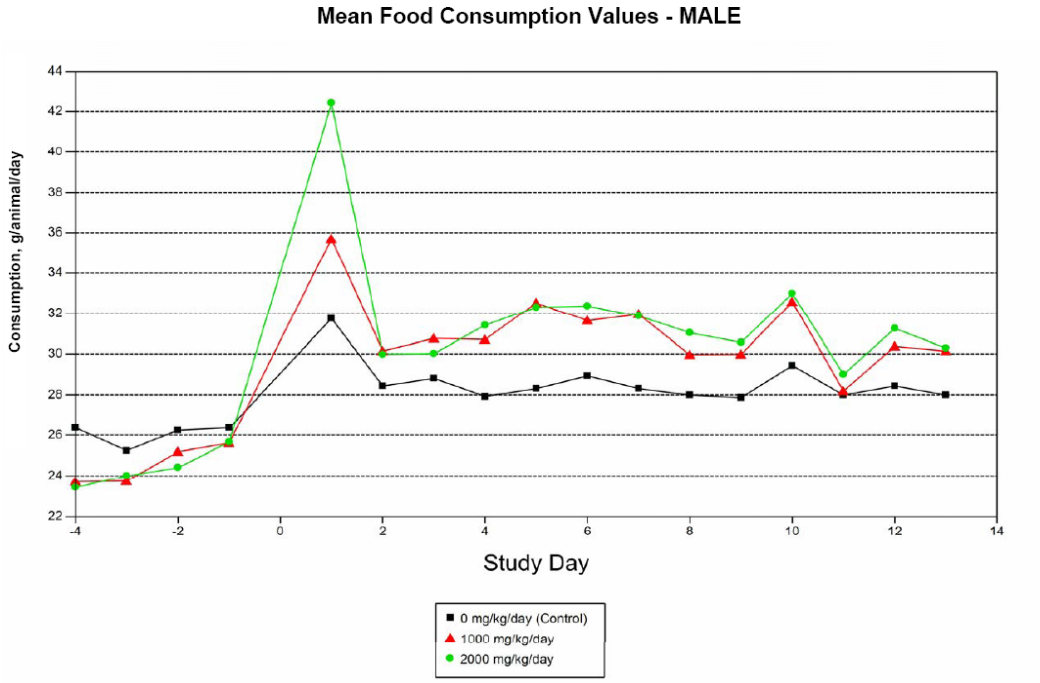
Methods

Doses:	0,1000,and 2000 mg/kg Gabapentin
Frequency of dosing:	Daily
Route of administration:	in diet
Formulation/Vehicle:	Lab Diet® Certified Rodent Diet#5002, PMI Nutrition International, Inc.
Species/Strain:	Male Wistar (Crl:WI) rats
Number/Sex/Group:	16, 48 and 48 per groups
Weight:	261 to 319 g
Unique study design:	stored frozen at -50 to -90ºC until shipment on dry ice to XenoPort, Inc. (Santa Clara, CA) for analysis. Gabapentin concentrations in plasma were estimated: gabapentin plasma concentrations (µg/mL) = 0.55 + 1.18 × gabapentin blood concentrations (µg/mL)
Deviation from study protocol:	None

Table 6.1. Toxicokinetic Parameters for Gabapentin in Blood and Plasma on Day 14 Following Repeated Oral Administration of Gabapentin in Diet for 14 Days in Male Wistar Rats

Matrix	Gabapentin Dose (mg/kg/day)	TK Parameters of Gabapentin		
		C_{max} (µg/mL)	T_{max} (hr)	AUC_{0-24} (µg*hr/mL)
Blood	1000	59.8	9.00	1090
	2000	79.7	9.00	1500
Plasma[a]	1000	71.1	9.00	1300
	2000	94.6	9.00	1780

[a] Gabapentin plasma concentrations were estimated from blood data using the following plasma to blood equation: gabapentin plasma concentrations (µg/mL) = 0.55 + 1.18 × gabapentin blood concentrations (µg/mL) (XenoPort Study Report XP100-TK).



7. Toxicokinetic Study of Gabapentin in Male Wistar Rats Given Gabapentin in Feed for 14 Days: Collection of Blood. Determination of Gabapentin Concentration in Rat Whole Blood (K2EDTA) Supernatant by LC-MS/MS Method.

Study no.:	2010N 105807
Conducting laboratory and location:	XenoPort, Inc. (Santa Clara, CA
Date of study initiation:	
GLP compliance:	
QA statement:	
Drug, lot #, and % purity:	

Key Study Findings: This report provides the bio-analytical data and supporting documentation from analysis of the study samples, together with standard curves and quality for study Nos 105806 and 105808.

Conclusion: Adequate.

Appendix B:
Expert Opinion- The relevance of rat pancreatic tumors to human risk

1. (b) (4)

1) Acinar cell neoplasm in rats is a result of nongenotoxic effects on the growth of spontaneously occurring focal hyperplastic acinar cell lesions in the rat pancreas.
2) Gabapentin enacarbil is a very weak promoter of rat pancreatic acinar cell tumors.
3) About 90 per cent of human ductal adenocarcinomas contain mutations of the K*ras* gene. K*ras* mutations are also found in a fraction of Pancreatic Intraepithelial Neoplasia lesions that increase with the grade (degree of dysplasia) of the lesions.
4) Acinar cell adenomas and carcinomas in rats do not contain K*ras* mutations in studies involving both gabapentin-treated rats and rats treated with a genotoxic carcinogen (azaserine)
Conclusion: The differences in tumor type, involvement of K*ras* mutation (ductal AdCa, not acinar), and preneoplastic changes in humans and rats support the view that the process of tumorigenesis in the rat exocrine pancreas is different than that in human exocrine pancreas. These observations provide multiple reasons to doubt the relevance of the acinar cell adenomas and carcinomas seen the toxicologic studies of gabapentin enacarbil in Wistar rats for human risk assessment.

A Short Review of Acinar to Ductal Metaplasia (ADM) in the Context of Carcinogenesis in the Pancreas (Dr. (b) (4)
1) ADM, but not Pancreatic Intraepithelial Neoplasia (PanIN) and Pancreatic Ductal Adenocarcinoma (PDA) have been seen in mouse models in which oncogenes other than K*ras* (c-*myc*, TGF-α) are expressed in acinar cells.
2) studies in mouse models suggest the hypothesis that expression of mutant K*ras* in human acinar cells might drive an ADM/PanIN/PDA pathway.
3) To date, ADM with progression to ductal adenocarcinoma has occurred only when mutant K*ras* is expressed in acinar cells. Several rat acinar cell carcinomas including the gabapentin-induced acinar cell neoplasms have been assayed for K*ras* mutation with negative results. The data reviewed above suggest that in the absence of K*ras* mutation, acinar cells do not give rise to ductal adenocarcinomas that is similar to the dominant pancreatic cancer in humans.

2. (b) (4)

1) The presence of a single carcinoma within any group, whether in the controls or at the high dose or any other dose, is well within historical Controls
2) An increase in incidence of pancreatic acinar cell tumors in male and female Wistar rats is limited to an increase in adenomas, but not statistically significantly increased.

3) Because of evolving differences in the strains over time, differences in Purina certified diets, systemic exposure at the different doses cannot necessarily be used to compare precise quantitative differences and thresholds between the positive and negative results in the different Gabapentin and Gabapentin enacarbil carcinogenicity studies.
4) The threshold for a tumorigenic response may reasonably be higher or lower than that reported for gabapentin.
5) The rat model itself with regard to pancreatic acinar cell carcinogenesis, is not relevant to the human situation. It is predicted with considerable confidence that no carcinogenic effect will occur in humans.

3. (b) (4)

1) The pathogenesis of gabapentin-induced pancreatic acinar cell neoplasms has not been elucidated, although direct mitogenesis appears not to be involved. An epigenetic mechanism is involved in the induction of the acinar cell lesions.
2) Carcinoma is a spontaneous neoplasm which occurs with greater frequency in male rats than in females.
3) No substance that enhances this tumor type has been associated with neoplasia of any type in humans, and rat pancreatic acinar cell neoplasm as the sole tumorigenic effect is considered to be of no or limited significance for human safety.
6) Based on the negative findings at 500 mg/kg GEn, marginal finding at 2000 mg/kg Gabapentin enacarbil (GEn) and absence of increased carcinomas with 1000 mg/kg gabapentin it is reasonable to assume that a gabapentin exposure (AUC) of 1290 µg.h/ml would be non-carcinogenic, whether provided by dosing with gabapentin or GEn.
7) “In conclusion, based on the finding of only pancreatic acinar cell neoplasms in the GEn rat bioassay, a likely epigenetic mode of action, and the dose response relationships for this neoplasm relative to human exposures, I conclude that GEn at the therapeutic dose does not represent a cancer risk to patients.”

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/s/

LUANN MCKINNEY
04/05/2011

LOIS M FREED
04/05/2011
Please see memo for comments.

Tertiary Pharmacology Review

By: Paul C. Brown, Ph.D., ODE Associate Director for Pharmacology and Toxicology, OND IO
NDA: 22-399
Submission date: 9/16/08; resubmitted 1/9/09
Drug: gabapentin encarbil
Sponsor: GlaxoSmithKline
Indication: Restless Leg Syndrome (RLS)
Reviewing Division: Division of Neurology Products

Introductory Comments:
Gabapentin encarbil is a prodrug of gabapentin. A complete nonclinical development program was conducted for gabapentin encarbil. The pharm/tox reviewer and supervisor have determined that this information is generally complete and adequate. The only significant nonclinical issue of concern is the carcinogenicity finding in rats.

The applicant completed two-year carcinogenicity studies in mice and rats by gavage. These studies were reviewed by the division and the Executive Carcinogenicity Assessment Committee. No drug-related tumors were observed in the mouse. Increases in pancreatic acinar cell hyperplasia, adenomas, and adenomas plus carcinomas were observed in rats. The increase was statistically significant in males at 2000 and 5000 mg/kg/day and in females at 5000 mg/kg/day. The pharm/tox supervisor has noted that an apparent increase in adenomas was also observed in males at the low dose of 500 mg/kg/day and an increase in pancreatic acinar cell hyperplasia was noted in females at 2000 mg/kg/day. This may suggest that the NOAEL for neoplastic or preneoplastic effects is lower than that at which a clear statistical increase in tumors was observed. The dose of 500 mg/kg/day was associated with an AUC in males that was only about 8 fold the human AUC at the clinical dose of 600 mg/day.

The supervisory review includes a discussion of the possible relevance of these tumor findings to humans. Currently, there appears to be inadequate information to establish that these tumors are not relevant to humans.

Carcinogenicity studies with gabapentin showed the same tumor finding in rats although only in males. Other differences between the findings with gabapentin and gabapentin encarbil suggest that the signal may be potentially stronger with gabapentin encarbil, although such comparisons are difficult to make in any quantitative way.

Conclusions:
I have discussed this NDA with the division pharm/tox supervisor and agree that the pancreatic tumors observed in the rat are a potential concern and that relevance to humans can not be dismissed at this time. I agree that this should

be considered in the risk:benefit analysis. If currently available data from previous human use of gabapentin or the current risk:benefit analysis do not support approval then the applicant may be able to support approval by providing additional clinical data or nonclinical data that explain the relevance of the tumors to humans. The nonclinical studies mostly likely to be of use would focus on delineating the mechanism by which gabapentin encarbil induces the tumors in rats and whether that mechanism is active in humans.

Application Type/Number	Submission Type/Number	Submitter Name	Product Name
NDA-22399	ORIG-1	GLAXO GROUP LTD DBA GLAXOSMITHKLINE	SOLZIRA

This is a representation of an electronic record that was signed electronically and this page is the manifestation of the electronic signature.

/s/

PAUL C BROWN
02/12/2010

MEMORANDUM

DEPARTMENT OF HEALTH & HUMAN SERVICES
Public Health Service
Food and Drug Administration

Division of Neurology Products (HFD-120)
Center for Drug Evaluation and Research

Date: February 5, 2010

From: Lois M. Freed, Ph.D.
Supervisory Pharmacologist

Subject: NDA 22-399 (XP13512, gabapentin enacarbil, HORIZANT®)

NDA 22-399 for XP13512 (HORIZANT™ ER tablets), a novel prodrug of gabapentin for treatment of moderate-to-severe Restless Leg Syndrome (RLS), was submitted by GlaxoSmithKline on September 15, 2008. However, the application was withdrawn (Amendment 000, November 11, 2008) because of electronic (clinical) dataset issues. The NDA was resubmitted on January 9, 2009 (Amendment 0004), and filed (Agency letter, 3/13/09); at the time of filing, no "potential review issues" had been identified. The PUDFA date was originally December 9, 2009; the goal date was extended by 3 months due to submission of a major amendment during the last 3 months of the review cycle.

Although XP13512 is a prodrug, it is considered a new chemical entity because of the covalent bond between the gabapentin and cycloxyalkoxycarbamate moieties. The sponsor conducted a full battery of nonclinical studies to support the NDA (under IND 71352; (b) (4) these studies have been reviewed by Terry S. Peters, D.V.M. (Pharmacology/Toxicology Review and Evaluation, 2/1/2010). Based on the review, Dr. Peters has concluded that the NDA "…is approvable from a nonclinical perspective" and recommends no additional nonclinical studies.

Summary of nonclinical findings

SOLZIRA® ER was developed in order to overcome the pharmacokinetic limitations of gabapentin (Neurontin; Park Davis [now Pfizer]), i.e., dose-related decreases in oral bioavailability, and short $t_{1/2}$ requiring multiple daily doses. Neurontin is approved for treatment of epilepsy (children and adults) and postherpetic neuralgia (adults), but not RLS.

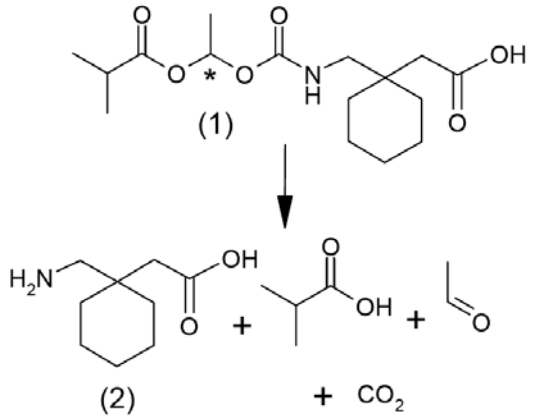
Pharmacology: The pharmacological activity of XP13512 responsible for efficacy in patients with RLS is unknown. XP13412 demonstrated no binding affinity when tested in

a battery of *in vitro* receptor and ion channel assays at a concentration of 10 μM. XP13512 was not tested in primary pharmacodynamic studies since, according to the sponsor, there is no animal model for RLS and since the pharmacological activity of XP13512 is presumed to reside solely in the gabapentin moiety. Gabapentin is a structural analog of GABA, but does not appear to interact with GABA receptors or modify GABA uptake or degradation. The mechanism(s) by which Neurontin exerts therapeutic effects in the indications for which it is approved is/are unknown.

PK/ADME: XP13512, a racemic mixture of R- and S- isomers, was designed to be stable in the GI tract and to be actively absorbed following oral dosing. (For quantitation of circulating levels of XP13512 and gabapentin, methanol was added to blood samples "as soon as possible after collection (typically within 5 minutes)…" in order to prevent post-collection hydrolysis of XP13512.)

In an investigative study in Sprague-Dawley rat, XP13512 was shown to be converted to gabapentin following i.v. and p.o. dosing. The GI and hepatic extraction ratios were estimated to be ≈80 and 54%, respectively. Therefore, following oral administration, the majority of the dose is presumed to be hydrolyzed to gabapentin during transport through GI tissues, not prior to absorption (sponsor's table below).

Figure 2.1. Structure of XP13512 and Hydrolysis to Gabapentin



Note: Chemical structure of XP13512 (1) and its enzymatic hydrolysis to release gabapentin (2), isobutyric acid, acetaldehyde, and carbon dioxide. *Denotes a chiral center.

In vitro studies in HEK cells and oocytes indicate involvement of the monocarboxylate transporter type-1 and the sodium dependent multivitamin transporter in the uptake of XP13512.

Acute dose studies in rat demonstrated similar conversion of the R-and S- enantiomers of XP13512 to gabapentin following oral (≈58 and 49%, respectively) and intracolonic (14 and 17%, respectively) administration, indicating lack of stereoselective conversion or absorption. In Sprague-Dawley rat, highest levels of radioactivity following a single oral dose of ^{14}C-XP13512 were detected in pancreas and kidney in both males and females; at 2-hrs post dose, tissue levels were 5- and 3-fold higher, respectively, than plasma levels.

There appeared to be no selective binding of radioactivity to pigmented tissues following acute oral dosing in Long-Evans rats. In rats, the primary route of elimination was urine (>90% of total radioactivity recovered in urine by 12 hours post dose).

In animals and human, XP13512 is hydrolyzed to gabapentin, isobutyric acid, acetaldehyde, and CO_2. There are no major circulating metabolites of gabapentin in humans; plasma levels of XP13512 are ≤2% of gabapentin levels at similar doses (cf. *Clinical Pharmacology/Biopharmaceutics Review, NDA 22-399*, Ju-Ping Lai, Ph.D., 8/11/2009). In animals, circulating levels (C_{max}, AUC) of XP13512 were <1% of gabapentin levels in mouse, rat, and rabbit, and <3-8% of gabapentin levels in monkey; therefore, from a PK/ADME standpoint, these animals are relevant models for predicting the safety of XP13512 in humans.

Toxicology: the pivotal oral toxicity studies were conducted in B6C3F1 mouse (13-wk dose-range finding, 2-year carcinogenicity), Wistar rat (acute, 2-, 13-, and 26-week general toxicity, 2-year carcinogenicity, reproductive and developmental toxicity), cynomolgus monkey (1-, 2-, 13-, and 39-week general toxicity), and New Zealand White rabbit (embryo-fetal development).

- **Repeat-dose toxicity studies**

Rat: XP13512 was administered by gavage at doses up to 5000 mg/kg/day for up to 26 weeks. CNS (altered motor activity [hypoactivity, hyperactivity]), liver (centrilobular hypertrophy), and kidney (in males; microscopic changes consistent with $\alpha_{2\mu}$-globulin nephropathy, but not confirmed for XP13512) were the apparent target organs. Plasma exposures achieved in the 26-week study at the HD of 5000 mg/kg were consistent with extensive hydrolysis of XP13512 to gabapentin. At Week 26, plasma C_{max} and AUC for gabapentin were ≈40-50 fold higher in rats than in humans at the sponsor's MRHD of 1200 mg QD; plasma C_{max} and AUC for XP13512 were ≈150-870 fold higher than in humans at 1200 mg QD.

Monkey: XP13512 was administered by gavage at doses up to 2000 mg/kg/day for up to 39 weeks; no justification appears to have been provided for high dose selection. No drug-related findings were observed in the 2-, 13-, or 39-week studies. In shorter term dose-range finding studies (acute, 7-day) in 1-2/sex/group, emesis and salivation were observed at ≥1000 mg/kg/day; in the 7-day study, transient decreases in food consumption and rbc parameters were observed at 500 and 2000 mg/kg. The NOAEL for monkey was consistently identified by the sponsor as >2000 mg/kg, even in the short-term studies; 5000 mg/kg was characterized as the "maximum practical dose". Based on these data, it is clear that drug-related toxicity was not fully characterized in the monkey.

In the 39-week study, mean plasma exposures (Week 39) at 2000 mg/kg/day to XP13512 and gabapentin were as follows:

DRUG	SEX	C_{max} (µg/mL)	$AUC_{(0-24\ hr)}$ (µg*hr/mL)	A:H RATIO*	
				C_{max}	AUC
gabapentin	M	357 ± 78.5	3190 ± 769	57	38
	F	375 ± 57.6	3560 ± 536	60	43
	total	366 ± 66.3	3370 ± 660	54	41
XP13512	M	11.9 ± 4.75	46.9 ± 13.8	661	1803
	F	15.6 ± 4.39	61.6 ± 6.30	867	2369
	total	13.7 ± 4.78	54.3 ± 12.8	761	2088

*plasma C_{max} and AUC following a single dose of 1200 mg QD (Study XP022) were 6.24 ± 1.55 µg/mL and 83.0 ± 21.8 µg*hr/mL, respectively, for gabapentin and 0.018µg/mL and 0.026 ± 0.018 µg*hr/mL, respectively, for XP13512.

Although the plasma AUC for gabapentin did not reach 50-fold that in humans at the MRHD of 1200 mg QD (cf. *ICH M3(R2) June 2009*) and dose-limiting toxicity was not observed, there is no clear need to repeat the study, taking into considering the extent of previous human experience with gabapentin and the large safety margins achieved with XP13512.

The plasma AUCs achieved for gabapentin (and XP13512) in monkeys provides a sufficient safety margin (>50-fold) compared to that in humans at a clinical dose of 600 mg/day.

- **Reproductive and developmental toxicology**

The sponsor conducted a full battery of reproductive and developmental toxicology studies of XP13512. XP13512 was tested at oral doses of 0, 200, 1000, and 5000 mg/kg in fertility and early embryonic development and embryo-fetal development studies in Sprague-Dawley rat and at doses of 0, 200, 500, and 2500 mg/kg in New Zealand White rabbit. These studies were adequate and demonstrated adverse effects on offspring pre- and post-natal development (e.g., reduced fetal and pup body weight relative to controls, decreased postnatal survival), but no evidence of teratogenicity. It is, however, somewhat surprising that no drug-related effects (e.g., delayed skeletal ossification) were evident upon fetal examination, considering the adverse effect on fetal body weight. XP13512 was report to have minimal, if any, effects on mating and fertility.

To support the NDA for Neurontin, reproductive and developmental toxicity studies were conducted in CD-1 mouse, Sprague-Dawley rat (fertility and general reproduction), Tif:F(SPF) rat (embryo-fetal development), and White Russian rabbit (Petrere JA, Anderson JA. *Fund Appl Toxicol* 23:585-589, 1994). While no teratogenicity was detected, fetal effects were detected that were not reported for XP13512. For example, "…delayed ossification of several bones in the skull, vertebrae, forelimbs, and hindlimbs" was observed in mice and "…increased incidence of hydroureter and/or hydronephrosis…" was observed in three separate (fertility and general reproductive performance, embryo-fetal development, and peri/postnatal) studies in rat (cf. *Package Insert*, approved 4/23/09; Petrere & Anderson, 1994). These findings for gabapentin may need further discussion when labeling is proposed for XP13512.

- **Genetic Toxicology**

The sponsor conducted a standard battery of genetic toxicology assays on XP13512 (i.e., *in vitro* Ames [3 studies], *in vitro* chromosomal aberration assay in HPL, *in vivo* micronucleus assay in rat [oral doses up to 2000 mg/kg]), as well as an *in vitro/in vivo* UDS assay in rat primary hepatocytes. XP13512 was negative in all but the *in vitro* chromosomal aberration assay (#RD2007/01489/00). In that assay, XP13512 was positive, both in the absence and presence of metabolic activation.

In study #RD2007/01489/00; XP13512 induced structural aberrations in the initial assay (3-hr treatment) at concentrations ≥1500 µg/mL without metabolic activation and at concentrations ≥1200 µg/mL with metabolic activation. In a confirmatory assay (3-hr treatment, with metabolic activation only), XP13512 induced structural aberrations at concentrations ≥500 µg/mL. Clear concentration-related positive responses, with and without metabolic activation, were observed in the absence of excessive cytotoxicity.

The sponsor attributed the clastogenic response to release of acetaldehyde (demonstrated to be genotoxic and carcinogenic, cf. Hengstler JG *et al*. *Annu Rev Pharmacol Toxicol* 43:485-520, 2003; Lambert B, He SM. *Ann NY Acad Sci* 534:369-376, 1988) resulting from the hydrolysis of XP13512 during the incubation period. The sponsor did not conduct definitive studies to document this possibility (e.g., testing XP13512 *in vitro* in the presence of aldehyde dehydrogenase). Instead, the sponsor conducted a study to quantitate the release of gabapentin following incubation of human whole blood cultures with XP13512 at concentrations of 1500 and 4500 µM (equivalent to 493 and 1480 µg/mL, respectively) over a 3-hour period under the assay conditions used in the *in vitro* genotoxicity assay (study #RD2008/00754/00XP020). Acetaldehyde was not measured directly, but was presumed to be released in equal molar amounts to gabapentin. The maximum concentrations of acetaldehyde released at 1500 and 4500 µg/mL (after 3 hrs incubation) were estimated to be similar to those at which increases in structural aberrations were detected.

Acetaldehyde has been reported to be "…at best only a weak mutagen in the standard *Salmonella* plate test…" (Norppa H *et al*. *Cancer Res* 45:4816-4821, 1985), but Norppa *et al*. (1985) note that "The low mutagenicity of acetaldehyde may be related to the poor sensitivity of the conventional *Salmonella* strains to cross-linking agents…", among other factors. Therefore, it is notable that none of *in vitro* Ames assays conducted for XP13512 included a tester strain to detect cross-linking mutagens, such as *S. typhimurium* TA102 or *E.coli* WP2 pKM101.

According to the sponsor, the positive clastogenicity observed with XP13512 due to formation of acetaldehyde is not a safety concern for a number of reasons, including the fact that circulating levels of acetaldehyde in humans following consumption of ethanol ("A standard alcoholic drink produces about 12.6 g of acetaldehyde.") are markedly higher than would result from a 1200-mg/day dose of XP13512. However, the most compelling argument is that at the doses of XP13512 tested in the 2-year carcinogenicity

study in mouse and rat, the estimated doses of acetaldehyde (13.4% of XP13512) were up to 500 and 250 times the dose in humans at 600 and 1200 mg/day, respectively, based on mg/kg, and ≈40-80 and 20-40 times the dose in humans at 600 and 1200 mg/day, respectively, based on mg/m^2, and the tumor profile was similar to that for gabapentin which is not converted to acetaldehyde.

To support the NDA for Neurontin, the following genetic toxicology assays were conducted: *in vitro* Ames assay, *in vitro* HGPRT forward mutation assay in CHL cells, *in vitro* chromosomal aberration assay in CHL cells, "*in vivo* chromosomal aberration assay and in the *in vivo* micronucleus test in Chinese hamster bone marrow", *in vivo* mouse micronucleus assay, UDS in rat hepatocytes. Gabapentin was negative in all assays in which it was tested (cf. *Package Insert*, approved 4/23/09; Radulovic LL *et al. Drugs Today* 31(8):P597-611, 1995).

Conclusion: XP13512 was negative in a standard battery of genetic toxicology assays, except for the *in vitro* chromosomal aberration assay in human lymphocytes. The sponsor attributed the clastogenic response to formation of acetaldehyde in the culture medium. Although the sponsor did not conduct studies to support this position, it is a reasonable hypothesis, given previous published studies of the genotoxic potential of acetaldehyde. Exposure to XP13512 and, potentially, to acetaldehyde was higher in the 2-year carcinogenicity studies in mouse and rat than that anticipated in humans at either 600 or 1200 mg/day. Therefore, the available *in vivo* data mitigate the concern regarding the *in vitro* clastogenic effect of XP13512.

Impurities

(b) (4)

Therefore, these impurities do not represent a safety concern.

- **Carcinogenicity**

Mouse: a 2-year carcinogenicity study was conducted at doses of 0, 500, 2000, and 5000 mg/kg/day; XP13512 was administered by gavage at doses up to the maximum feasible dose. Survival rates were dose-dependently reduced in males compared to control (survival rates at Wk 105: 83, 70, 63, and 50%, respectively). However, as Dr. Peters notes, there was a sufficient number of survivors for a sufficient duration to ensure an adequate study. Survival was not adversely affected in females. No drug-related tumors were detected (cf. *Executive CAC meeting minutes, 8/5/09*).

To support the NDA for Neurontin, a 104-week oral (dietary) carcinogenicity study was conducted in mice at doses of 200, 600, and 2000 mg/kg/day. No tumor findings were reported (cf. *Package Insert*, approved 4/23/09).

Rat: a 2-year carcinogenicity study was conducted at doses of 0, 500, 2000, and 5000 mg/kg/day; XP13512 was administered by gavage at doses up to the maximum feasible dose. There was an increase in mortality in MDM and HDM, resulting in early termination of those groups (Weeks 97 and 90, respectively). Survival was not affected in females. The incidence of pancreatic acinar cell hyperplasia and tumors was increased in males and females; the data are summarized in the following table:

FINDING	MALES				FEMALES			
	0	**500**	**2000**	**5000**	**0**	**500**	**2000**	**5000**
NEOPLASTIC								
adenoma	2/60	4/60	4/60	8/60	0/60	0/60	0/60	3/60
carcinoma	0/60	0/60	1/60	1/60	0/60	0/60	0/60	1/60
total	**2/60**	**4/60**	**5/60**	**9/60**	**0/60**	**0/60**	**0/60**	**4/60**
NON-NEOPLASTIC								
hyperplasia								
minimal	8/60	2/60	4/60	5/60	1/60	0/60	2/60	5/60
mild	3/60	6/60	7/60	12/60	0/60	0/60	1/60	5/60
moderate	3/60	1/60	3/60	3/60	0/60	1/60	1/60	4/60
severe	0/60	1/60	0/60	0/60	0/60	0/60	0/60	0/60
total	**14/60**	**10/60**	**14/60**	**20/60**	**1/60**	**1/60**	**4/60**	**14/60**

According to the Pathologist's Report (Laura S. Zwick, DVM, DACVP), "…given the lower survival rate and earlier termination date of males at 2000 and 5000 mg/kg/day compared to control animals, it is possible that the incidence and severity of acinar cell hyperplasia and neoplasia would have occurred at an even higher rate at these dose levels if these males had survived as long as the control males." The Pathologist's Report also noted that the incidence of pancreatic acinar cell adenomas in HDM exceeded the historical control (HC) range (0-8.3%) for the laboratory. (The sponsor provided no other information regarding the HC data for tumor incidence.)

Following review of the data by the ExeCAC, it was concluded that "There was an increased incidence of pancreatic acinar cell hyperplasia, adenomas, and adenomas + carcinomas in males and females at the HD and in MDM" (cf. *Executive CAC meeting minute*s, 8/5/09). The ExeCAC did not consider the small increase in hyperplasia in mid-dose females to be drug-related. However, considering the fact that the only finding in control females was minimal hyperplasia in a single animal and the clear increase in tumors and hyperplasia at the high dose, one could argue that the increased incidence and severity of hyperplasia in mid-dose females reflect a drug-related increase in pre-neoplastic changes at that dose. According to the study pathologist, "The incidence and/or severity of acinar cell hyperplasia in both sexes at 2000 mg/kg/day were slightly increased compared to controls, and, although uncertain, these changes may have also been treatment related."

TK data were not collected in the 2-year study. Based on the TK data from the 26-week study (provided in the sponsor's table below), conducted using the same oral (gavage) doses, the safety margins between the plasma AUC achieved at the low dose in males and the mid dose in females (i.e., doses not clearly associated with pancreatic acinar cell hyperplasia and tumors) and that in humans at the clinical dose of 600 mg/day are ≈8 and 30-fold, respectively. The safety margins at the clinical dose of 1200 mg/day are ≈4 and 14-fold, respectively. Based on these safety margins, the pancreatic acinar cell findings should be considered clinically relevant.

Table 4.2 Toxicokinetic Parameters for Gabapentin in Blood of Rats After Oral Dosing of XP13512 – DAY 175

Group	Dose	Gender	C_{max} (µg/mL)	T_{max} (hr)	$T_{1/2}$ (hr)	AUC_{last} (µg*hr/mL)	$AUC_{(0-24)}$ (µg*hr/mL)
2	500 mg/kg/day	F	103	1.00	2.22	317	317
		M	107	1.00	2.59	407	407
		Combined Ave.	105	1.00	2.40	362	362
3	2000 mg/kg/day	F	211	2.00	2.57	1470	1470
		M	201	1.00	3.81	1470	1470
		Combined Ave.	206	1.50	3.19	1470	1470
4	5000 mg/kg/day	F	320	1.00	4.47	3020	3020
		M	371	4.00	4.79	3230	3230
		Combined Ave.	345	2.50	4.63	3130	3130

[According to the data from clinical trial #XP081, the plasma gabapentin $AUC_{ss,24}$ in humans following multiple oral doses of XP13512 in the fed state was 51.4 µg•hr/mL at 600 mg/day and 95.7 µg•hr/mL at 1200 mg/day.]

To support the NDA for Neurontin, a 104-week oral (dietary) carcinogenicity study of gabapentin was conducted in rats at doses of 250, 1000, and 2000 mg/kg/day. "A statistically significant increase in the incidence of pancreatic acinar cell adenomas and carcinomas" was reported in HDM (cf. *Package Insert*, approved 4/23/09). The results of this study were published by Sigler *et al*. (Sigler RE *et al. Toxicology* 98:73-82, 1995); the reported microscopic findings in pancreatic acinar cells of males are summarized in the following table:

FINDING	DOSE (mg/kg)			
	0	**250**	**1000**	**2000**
hyperplasia	21/50	22/50	20/50	23/50
adenoma	7/50	6/50	10/50	16/50
carcinoma	0/50	4/50	3/50	8/50

Based on published data (Radulovic LL *et al*. *Drugs Today* 31(8):P597-611, 1995; Sigler RE *et al*., 1995) and labeling (*Package Insert*, 4/23/09) for gabapentin, there appear to be several potential differences between the carcinogenicity findings for gabapentin and XP13512 in rat:

- Pancreatic acinar cell adenomas and carcinomas were observed in both males and females with XP13512, whereas these tumors were observed only in males treated with gabapentin. [It is of note that the spontaneous incidence of pre-neoplastic

and neoplastic changes in pancreatic acinar cells was lower in females than in males.]

- A statistically significant increase in the incidence of pancreatic acinar cell adenomas and carcinomas was observed in XP13512-treated males at the mid and high doses, but reported only at the high dose in males treated with gabapentin. [For Neurontin, the ExeCAC and the sponsor concluded that the only drug-related effect was in high-dose males; however, considering the fact that no carcinomas were reported in control males, it could be argued (in the absence of relevant historical control data indicating that the control incidence was spuriously low) that a drug-related effect was also observed in low and mid-dose males.]
- The pancreatic acinar cell carcinomas detected in XP13512-treated males and females were described in the study pathologist's report as "locally invasive without evidence of distant metastases"; the acinar cell carcinoma was the cause of death in the affected mid-dose male, but not in the high-dose animals. The pancreatic acinar cell tumors reported for gabapentin were "…considered low grade since they did not invade adjacent tissues, metastasize or cause the death of any animal…" (Radulovic *et al*., 1995).
- The incidence of pancreatic acinar cell hyperplasia was dose-related in males and females treated with XP13512, whereas with gabapentin, the incidence of hyperplasia was similar among groups in males.

These differences, although notable, do not necessarily indicate a fundamental difference in tumor profile between XP13512 and gabapentin. Rather, the data for XP13512 may provide additional characterization of the carcinogenic potential of gabapentin. It is difficult to directly compare the results of these studies, for several reasons. For example, the method of dosing was different (dietary vs gavage) and a direct comparison of exposure cannot be made due to the lack of available TK data for the gabapentin study. Simply based on relative doses of gabapentin (i.e., gabapentin accounts for ≈52% of XP13512 on a weight basis), the doses of XP13512 used in the carcinogenicity study would correspond to gabapentin doses of 260, 1040, and 2600 mg/kg; however, due to the reportedly poor absorption of gabapentin administered orally, one cannot assume that plasma exposures were comparable, even though doses of gabapentin tested in the two studies were fairly similar. Nonetheless, the 2-year carcinogenicity study of XP13512 in rats confirms the association between gabapentin and pancreatic acinar adenomas and carcinomas, and suggests a more aggressive tumor (locally invasive and lethal carcinoma in one animal) than did the findings for gabapentin (no locally invasive or lethal carcinoma in any animal). Also, the 2-year study of XP13512 in rat confirms the observation with gabapentin that the spontaneous incidence of pancreatic acinar cell tumors is lower in female than in male rats. Therefore, the increase in these tumors in treated females suggests that XP13512 (and presumably gabapentin) may not simply exacerbate a spontaneous finding.

A series of investigative studies was conducted for Neurontin in an attempt to identify the mode of action for the gabapentin-induced pancreatic tumors. Gabapentin is presumed to exert its effect via an epigenetic mechanism, based on the demonstrated lack of genotoxic potential in a standard battery of genetic toxicology assays. Other agents associated with

pancreatic acinar cell tumors (e.g., raw soy flour, azaserine) are thought to act by increasing the sensitivity to CCK, possibly by enhanced expression of CCK receptors in the rat pancreas. This mode of action could not be demonstrated for gabapentin (cf. Dethloff L *et al. Toxicol Sci* 55:52-59, 2000). According to Dethloff *et al*. (2000), "…we were unable to discern changes in CCK concentrations or in CCK receptor populations in pancreas of gabapentin-treated rats…", although there appeared to be some question as to the validity of the methodology used. Dethloff *et al* (2000) also reported that "…gabapentin had no mitogenic effects demonstrable *in vivo*…", but some evidence of stimulation of DNA synthesis was obtained *in vitro*. The authors concluded that gabapentin might "behave as a weak tumor promoter", possibly by affecting intracellular calcium mobilization. While the mode of action has clearly not been established, the *in vitro* data suggest that gabapentin might induce pancreatic tumors through a pathway relevant to all species, including human. Due to the concern raised by the *in vitro* data, the following language was added to the Neurontin labeling:

> Studies designed to investigate the mechanism of gabapentin-induced pancreatic carcinogenesis in rats indicate that gabapentin stimulates DNA synthesis in rat pancreatic acinar cells *in vitro* and, thus, may be acting as a tumor promoter by enhancing mitogenic activity. It is not known whether gabapentin has the ability to increase cell proliferation in other cell types or in other species, including humans.

The sponsor did not conduct any mechanistic studies to further investigate the pancreatic tumor findings, but instead noted the approval of Neurontin. It is clear that "similar" findings did not preclude approval of gabapentin for postherpetic neuralgia or epilepsy. How RLS compares with these approved indications is a clinical issue. There are currently two approved therapies for RLS, Mirapex (pramipexole) and Requip (ropinirole). According to current labeling, pramipexole was negative in 2-year carcinogenicity studies in mouse and rat; ropinirole produced significant increases in testicular Leydig cell adenomas (all doses tested) in male rats and benign uterine endometrial polyps in female mice at the highest dose tested (10 times the maximum recommended human dose on a mg/m^2 basis). One other drug product, (b) (4)

(b) (4)

According to the most recent labeling

Therefore, compared to the approved drug products for treatment of RLS, the tumor signal for gabapentin and XP13512 appears to be of more concern, at least from a nonclinical standpoint.

A keyword search of the PDR online identified two drugs approved for treatment of hypercholesterolemia and hyperlipidemia, Welchol (colesevelam HCl) and Tricor (fenofibrate), associated with pancreatic acinar cell tumors. Welchol, a bile acid sequestrant, was tested in 104-week carcinogenicity studies in CD-1 mouse and Sprague-Dawley rat. No drug-related tumors were reported in mouse. In rat,

> "…a statistically significant increase in the incidence of pancreatic acinar cell adenoma was seen in male rats at doses >1.2 g/kg/day (approximately 20 times the maximum human dose, based on body weight, mg/kg) (trend test only)…" (*Package Insert*, approved 10/2/09).

Tricor, a lipid regulating agent, was tested in 21- and 18-month carcinogenicity studies in mouse, and two 24-month carcinogenicity studies and a 117-week carcinogenicity study in rat. Pancreatic tumors were not reported in mice. In rat studies,

> "In the first 24-month study…A statistically significant increase in pancreatic carcinomas was observed in males at 1 and 6 times the MRHD; an increase in pancreatic adenomas….was observed at 6 times the MRHD in males. In a second 24-month study in a different strain of rats, doses of 10 and 60 mg/kg/day (0.3 and 2 times the MRHD based on mg/meter^2 surface area) produced significant increases in the incidence of pancreatic acinar adenomas in both sexes…."
>
> "A 117-week carcinogenicity study was conducted in rats comparing three drugs: fenofibrate 10 and 60 mg/kg/day (0.3 and 2 times the MRHD), clofibrate (400 mg/kg/day; 2 times the human dose), and Gemfibrozil (250 mg/kg/day; 2 times the human dose) (multiples based on mg/ meter^2 surface area). Fenofibrate increased pancreatic acinar adenomas in both sexes. Clofibrate increased….pancreatic acinar adenomas in males…"
>
> (Other tumor findings were reported; only those related to pancreatic acinar cells are provided above; *Package Insert*, approved 11/5/04.)

Welchol produced pancreatic acinar cell adenomas, but no carcinomas, in males at doses above the maximum human dose. Tricor produced pancreatic carcinomas in one of three studies; however, the tumor site was not specified. Tricor did produce pancreatic acinar cell adenomas in males and females in two separate studies, at clinically relevant doses. Clofibrate was also reported to increase adenomas, but only in males; how the doses tested compared to clinical doses was not noted. (Clofibrate is no longer marketed in the U.S.) It is difficult to compare the findings for these drug products without access to the original data, but it is of note that findings of pancreatic adenomas (and possibly carcinoma) were reported for two marketed products. How the indications for which they are approved and the clinical benefit of these products compared with XP13512 is a clinical issue. Another consideration is the potential mechanism underlying the pancreatic findings for Welchol and Tricor; no discussion of potential mechanism(s) was provided in labeling for either drug product. It is unknown if these drugs and XP13512 (and gabapentin) induce pancreatic tumors through similar or different mechanisms.

Relevance of gabapentin-induced pancreatic tumors to humans has been questioned based on several considerations, including that the tumor findings appear species specific and that the rat pancreas may not be an adequate model for human pancreatic cancer. Neither of these possibilities has been documented; however, it is possible that additional

nonclinical (including investigative studies) or clinical information may mitigate the concern regarding these tumors. Gabapentin was approved in 1993 for treatment of epilepsy and in 2002 for treatment of postherpetic neuralgia; therefore, there is extensive human experience, much of it involving chronic administration at higher doses or exposures. Whether or not the clinical benefit and/or the previous human experience, support approval of XP13512 in light of the potential carcinogenic risk identified in the rat is a clinical decision. If, after taking these into consideration, concern remains, the sponsor should be asked to provide additional information to address the carcinogenic potential of XP13512 and its relevance to humans prior to approval.

Recommendation

As concluded by Dr. Peters, the sponsor has conducted a complete and adequate non-clinical assessment of XP13512 to support an NDA for the treatment of RLS. There is, however, concern that the pancreatic acinar cell tumors observed with XP13512, which confirm the same findings observed with gabapentin, represent a carcinogenic risk to humans. If the available human experience with gabapentin and/or the anticipated clinical benefit of XP13512 do not support approval, considering this risk, then the sponsor should provide additional nonclinical (including investigative studies) or clinical data to demonstrate a lack of clinical relevance for the pancreatic tumors observed in rat.

Labeling

It is my understanding that labeling is not being considered at this time.

Application Type/Number	Submission Type/Number	Submitter Name	Product Name
NDA-22399	ORIG-1	GLAXO GROUP LTD DBA GLAXOSMITHKLINE	SOLZIRA

This is a representation of an electronic record that was signed electronically and this page is the manifestation of the electronic signature.

/s/

LOIS M FREED
02/05/2010

DEPARTMENT OF HEALTH AND HUMAN SERVICES
PUBLIC HEALTH SERVICE
FOOD AND DRUG ADMINISTRATION
CENTER FOR DRUG EVALUATION AND RESEARCH

PHARMACOLOGY/TOXICOLOGY REVIEW AND EVALUATION

NDA NUMBER:	**22-399**
SERIAL NUMBER:	**000**
DATE RECEIVED BY CENTER:	**9/16/08; resubmitted 1/9/09**
PRODUCT:	G**abapentin enacarbil extended release tablets** (HORIZANT®)
INTENDED CLINICAL POPULATION:	**Patients with moderate to severe Restless Legs Syndrome**
SPONSOR:	**GlaxoSmithKline**
DOCUMENTS REVIEWED:	**Electronic submission**
REVIEW DIVISION:	**Division of Neurology Products**
PHARM/TOX REVIEWER:	**Terry S. Peters, D.V.M.**
PHARM/TOX SUPERVISOR:	**Lois M. Freed, Ph.D.**
DIVISION DIRECTOR:	**Russell Katz, M.D.**
PROJECT MANAGER:	**Beverly Connor**

TABLE OF CONTENTS

EXECUTIVE SUMMARY

I. Recommendations

A. Recommendation on approvability
XP13512 is approvable from a nonclinical perspective.

B. Recommendation for nonclinical studies
Further mechanistic information concerning the positive pancreatic neoplastic findings in the rat carcinogenicity study would be useful in helping determine the human risk from HORIZANT®. The pancreatic tumors are similar to those elicited with gabapentin (tumors were found in female rats with HORIZANT® but not with gabapentin) but the indication of "Restless Legs Syndrome" does not warrant the same risk: benefit assessment as epilepsy or post-herpetic neuralgia.

C. Recommendations on labeling:

Label content as of 10/9/09:
Lines 124- 136:

5.5 Tumorigenic Potential

(b) (4)

The doses administered to rats and mice should be converted to mg/kg/d. The animals' multiples of exposure are 7 times (500 mg/kg/d dose), 28 times (2000 mg/kg/d dose) or 61 times (5000 mg/kg/d dose) the human exposure at 600 mg/day (AUC steady state: 51.4 µg.h/mL) and 3.8 times (500 mg/kg/d dose), 15.3 times (2000 mg/kg/d dose) or 32.7 times (5000 mg/kg/d dose) the human exposure at 1200 mg/day (AUC steady state: 95.7 µg.h/mL), respectively. The multiples should be "28 or 61 times, respectively, the human gabapentin exposure at a dose of 600 mg/day."

Lines 381- 407:
Doses should be converted to mg/kg/d. Exposure margins should be consistent with Lines 124- 136.

Lines 403- 407: The following statement should be added: Rabbit maternal toxicity was shown by adverse clinical signs, decreased body weights and premature parturition

evident in all studies. Embryo-fetal toxicity was found in rat pups at 5000 mg/kg/d and rabbit kits at 2500 mg/kg/d.

II. Summary of nonclinical findings

A. Brief overview of nonclinical findings

XP13512 is a pro-drug of gabapentin (a gamma-aminobutyric acid analog) that the sponsor developed to provide a more reliably available and sustained exposure than that achieved with gabapentin (marketed as Neurontin® by Pfizer).

In the safety pharmacology studies, single oral doses of XP13512 elicited hypoactivity, ataxia and decreased muscle tone in rats treated with ≥250 mg/kg. These signs were noted within 30 minutes of dosing and were reversed within 24 hrs of dosing. No adverse effects on respiratory parameters were noted in rats at single oral doses up to 750 mg/kg. In repeated dose oral studies in rats, increased and/or decreased motor activity was found in males at doses up to 2000 mg/kg/d but not at 5000 mg/kg/d.

The in vitro hERG assay showed no effects at concentrations ≤100 µM. Monkeys treated for up to 39 weeks with oral doses ≤2000 mg/kg/d did not demonstrate any significant adverse cardiovascular effects.

Pharmacokinetic studies were performed primarily via the oral route in mice, rats, rabbits and cynomolgus monkeys. Plasma exposures to XP13512 were low in all species tested and rapid hydrolysis to gabapentin was measured. Oral bioavailability to gabapentin was high in rats and monkeys, in contrast to bioavailability after gabapentin administration where a dose-dependent decreased bioavailability was determined. In repeated dose studies in mice, rats and monkeys, C_{max} gabapentin values increased proportionally with dose in monkeys but less than dose-proportionally in mice and rats. Mean AUC values for gabapentin after administration of XP13512 increased dose-proportionally in all species. No significant gender-related differences were appreciated.

XP13512 is moderately bound to human serum albumin. The test article appears to be transported across human Caco-2 cells, indicative of active transport. It does not appear to be a substrate of human P-glycoprotein but does appear to be a substrate for monocarboxylate transporter type-1 (MCT-1) and sodium dependent multivitamin transporter (SMVT) in vitro. In *in vitro* studies, the test article did not serve as a substrate or an inhibitor for human cytochrome P450 enzymes.

Distribution of radiolabeled XP13512 in albino and pigmented rats showed radioactivity at highest levels in the pancreas and kidney at 2 hrs post-dosing and essentially no radiolabel at 24 hrs post-dosing. The majority of the radiolabel was recovered in urine, primarily in the first 12 hrs.

Repeated dose testing via the oral route was performed in several species: up to 26 weeks in albino rats at doses up to 5000 mg/kg/d, up to 39 weeks in cynomolgus monkeys at doses up to 2000 mg/kg/d. In rats, the doses were 0, 500, 2000 or 5000 mg/kg/d. As in the shorter term rat studies, increased age-related chronic progressive nephropathy with hyaline droplet formation was noted in all treated male groups. Reversal was incomplete at the end of the recovery period. Centrilobular hepatocellular hypertrophy was described in the high dose animals but was reversed by the end of the 1 month recovery period. No NOAEL for the histologic renal findings was found in this study, but clinical chemistries and urinalyses were not affected. Cynomolgus monkeys were treated by oral gavage with 0, 250, 1000 or 2000 mg/kg/d of XP13512. No adverse effects of treatment were found in any of the parameters evaluated. The NOEL is determined to be 2000 mg/kg/d. Plasma exposures (AUC) to gabapentin at the highest dose were 3370 µg.h/mL at the end of the 9 month period while exposures to XP13512 were 54.3 µg.h/mL at the same time point (1.6% of the gabapentin level), demonstrating essentially complete hydrolysis of the test article to gabapentin. The associated C_{max} values were 366 µg/mL and 13.7 µg/mL, respectively.

In the mouse 2-year carcinogenicity study, no increases in any tumor type were detected. In mice treated with 0, 500, 2000 or 5000 mg/kg/d by oral gavage, XP13512 treatment caused decreased survival in the mid and high dose males and increased body weights in the high dose animals. No other significant findings were appreciated in treated animals except for a modest exacerbation of age-related axonal/myelin degeneration of the sciatic nerve in the females at 2000 mg/kg/d and both sexes at 5000 mg/kg/d.

In the rat carcinogenicity study, Wistar rats were treated for up to 104 weeks with 0, 500, 2000 or 5000 mg/kg/d of XP13512 in Tween 80 and methylcellulose by oral gavage. The 2000 and 5000 mg/kg/d males were terminated early (Weeks 97 and 90, respectively) due to exacerbation of chronic progressive nephropathy. Females were not similarly affected. There was an increased incidence of pancreatic acinar cell hyperplasia, adenomas and carcinomas in both sexes at 5000 mg/kg/d and in males at 2000 mg/kg/d. The decreased survival and early termination in the 2000 and 5000 mg/kg/d males may be responsible for a lesser incidence of both non-neoplastic and neoplastic lesions. Thus, XP13512 is considered a carcinogen in rats under the conditions of this study.

XP13512 was not genotoxic in multiple Ames assays, the in vivo micronucleus or the UDS assays. However, it was positive in the in vitro chromosomal aberration assay in human lymphocytes. The etiology of this finding is reportedly (per sponsor) the release of acetaldehyde during the (b) (4). (b) (4) potential (b) (4) impurities were found to be genotoxic in the Ames assays, but the levels in the final product are below the level of concern.

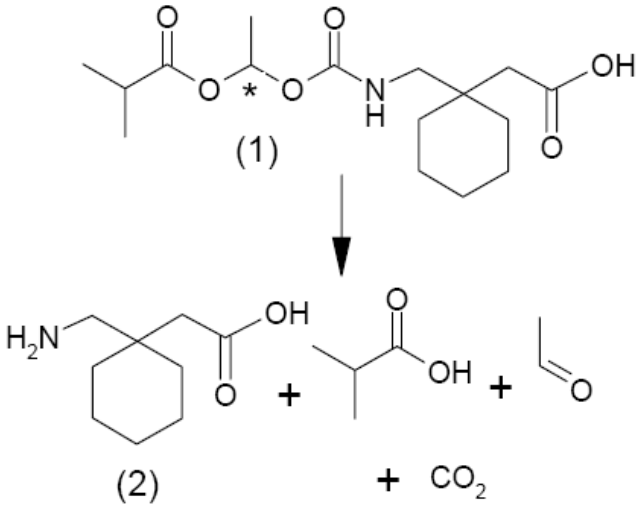
A complete battery of reproductive toxicity testing was conducted in rats and rabbits and no adverse effects were found on fertility or embryofetal development

parameters. Maternal toxicity was shown by adverse clinical signs, decreased body weights and premature parturition (rabbits only), which were evident in all species tested. Embryo-fetal toxicity was found in rat pups at 5000 mg/kg/d and rabbit kits at 2500 mg/kg/d.

XP13512 was not shown to be a skin sensitizer and it was a non-irritant on skin. It was found to be an ocular irritant.

B. Pharmacologic activity
XP13512 is rapidly and extensively hydrolyzed in vitro and in vivo by non-specific esterases to gabapentin and its pharmacodynamic activity is attributable to gabapentin.

Figure 2.1. Structure of XP13512 and Hydrolysis to Gabapentin



Note: Chemical structure of XP13512 (1) and its enzymatic hydrolysis to release gabapentin (2), isobutyric acid, acetaldehyde, and carbon dioxide. *Denotes a chiral center.

C. Nonclinical safety issues relevant to clinical use
The primary issues of concern are:

1) XP13512 is primarily excreted in the urine of nonclinical species and its administration exacerbated age-related chronic progressive nephropathy.
2) Embryo-fetal toxicity was found in rat pups and rabbit kits
3) Pancreatic hyperplasia, adenoma and carcinoma increased in rats treated with ≥2000 mg/kg/d. The plasma exposure (AUC) multiple at the non-carcinogenic dose is approximately 4.3x the human clinical exposure. Gabapentin elicited the same tumors in rats at doses providing exposures of 6.5x the human clinical exposure.

Sponsor table:

Table 8.1. Gabapentin Exposure Following Oral Administration of XP13512: Comparative Systemic Exposure in Mice, Rats, Rabbits, Monkeys and Humans

Study Type Study No. (Report No.)	Dose of XP13512 (mg/kg/day)	Gabapentin Toxicokinetic Data			
		AUC_{0-24}[a] (µg.h/mL)	Multiple of Clinical Exposure[b]	C_{max}[a] (µg/mL)	Multiple of Clinical Exposure[b]
Repeat Dose Studies					
Mouse 13 week XP024 (RD2008/00324/00)	500	109	1.31	48.6	7.79
	2000	591	7.12	256	41.0
	5000 (NOAEL)	**1620**	**19.5**	**238**	**38.1**
Rat 13 week XP025 (RD2007/01523/00)	500	462	5.57	141	22.6
	2000 (NOAEL)	**1590**	**19.2**	**252**	**40.4**
	5000	5290	63.7	386	61.9
Rat 26 week XP046 (RD2007/01526/00)	500	362	4.36	105	16.8
	2000 (NOAEL)	**1470**	**17.7**	**206**	**33.0**
	5000	3130	37.7	345	55.3
Monkey 13 week XP026 (RD2007/01524/00)	500	775	9.34	115	18.4
	1000	1550	18.7	231	37.0
	2000 (NOAEL)	**2670**	**32.2**	**278**	**44.6**
Monkey 39 week XP047 (RD2007/01528/00)	250	316	3.81	52.2	8.37
	1000	1600	19.3	211	33.8
	2000 (NOAEL)	**2860**	**34.5**	**329**	**52.7**
Reproductive and Developmental Studies					
Rat EFD XP032 (RD2008/00144/00)	200	195	2.35	55.5	8.89
	1000 (NOAEL)	**988**	**11.9**	**166**	**26.6**
	5000	3310	39.9	302	48.4
Rabbit EFD XP034 (RD2008/00146/00)	200	311	3.75	105	16.8
	500	944	11.4	253	40.5
	2500 (NOAEL)	**3040**	**36.6**	**393**	**63.0**

2.6 PHARMACOLOGY/TOXICOLOGY REVIEW

2.6.1 INTRODUCTION AND DRUG HISTORY

NDA number: 22-399
Review number: 1
Sequence number/date/type of submission: N000; 9/15/08; Original NDA
Information to sponsor: Yes ( ) No (x)
Sponsor and/or agent: GlaxoSmithKline, Philadelphia, PA
Manufacturer for drug substance: (b) (4)
Reviewer name: Terry S. Peters, D.V.M.
Division name: Neurology Products
Review completion date: 2/1/10

Drug:

Trade name: HORIZANT®

Generic name: Gabapentin enacarbil

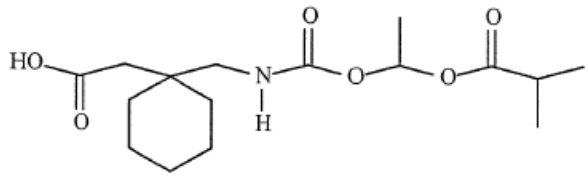
Code name: XP13512 (XenoPort, Inc. identification); GSK1838262 (GSK identification)

Chemical name: ((±-1-([(α-isobutanoyloxyethoxy)carbonyl]-aminomethyl)-1-cyclohexane acetic acid

CAS registry number: 478296-72-9

Molecular formula/molecular weight: $C_{16}H_{27}NO_6$; 329.40 g/mol

Structure:



Relevant INDs/NDAs/DMFs: (b) (4) IND 71,352 (current application): (b) (4)

Drug class: Novel prodrug of gabapentin

Intended clinical population: Patients with moderate to severe primary Restless Legs Syndrome

Clinical formulation: Extended release tablets designed to overcome the saturable oral bioavailability of gabapentin. The prodrug contains (b) (4)

Route of administration: Oral

Disclaimer: Tabular and graphical information are constructed by the sponsor unless cited otherwise.

Studies reviewed within this submission:
See tabulated summaries below at the end of the pertinent sections
Studies not reviewed within this submission:
1) RD2008/00262: LeadProfilingScreen Data Report as only summary data were provided2) Validation methods for LC/MS-MS Methods in mouse, rat, rabbit and monkey blood
3) Single dose toxicity studies in rats and non-human primates as the MTD/MFD doses identified in these studies were utilized in the repeat-dose toxicity studies

2.6.2 PHARMACOLOGY

2.6.2.1 Brief summary

The sponsor did not conduct any primary pharmacodynamic studies with XP13512. This product has no detectable pharmacologic activity until it has been hydrolyzed to gabapentin.

2.6.2.2 Primary pharmacodynamics

Mechanism of action: XP13512 is hydrolyzed to gabapentin by non-specific esterases in the intestine; therefore, the sponsor did not conduct any primary pharmacodynamic studies. Gabapentin binds with high affinity to the ά2δ subunit of voltage-gated calcium channels in the CNS. The hypothesis is that the therapeutic effects may be mediated by binding to the subunit eliciting a modulation of neurotransmission and reduction in excessive neurotransmitter release.

Drug activity related to proposed indication: The etiology of Restless Legs Syndrome is unknown at this time. No specific mechanism of action is proposed. No animal studies for Restless Legs Syndrome (RLS) were conducted due to these two factors.

2.6.2.3 Secondary pharmacodynamics

A single secondary pharmacology study (RD2008/00262/00XP091) conducted by (b) (4) was conducted to assess the potential for XP13512 to interact with ion channels and specific receptors. Summary data only were provided. It appears that XP13512 (10 µM) has low affinity for neurotransmitter receptors and did not bind to any extent to any of the receptors evaluated.

2.6.2.4 Safety pharmacology

Neurological effects:
XP13512: Effects on General Activity and Behaviour in the Rat Following Oral Administration; Study #RD2007/01533/00XP010 or 2337/001-D6146. This study was conducted by (b) (4) and was initiated on 6/16/03.

Wistar rats received XP13512 (0 [0.5% methylcellulose, 0.1% Tween 80], 75, 250 or 750 mg/kg) as a single oral dose in 7.5 mL/kg. An Irwin screen was performed at 30, 60, 90, 150 and 300 minutes post-dosing on Day 1.

Results: Treatment at 30 mg/kg of XP13512 did not elicit any significant adverse effects.

With dosing at 250 and 750 mg/kg, hypoactivity, ataxia, hyposensitivity to touch, and apathy were noted within 60 min (250 mg/kg) or 30 minutes (750 mg/kg) of dosing. Signs were mild in most animals at 250 mg/kg, with females appearing normal within 6 hrs of dosing. At 750 mg/kg, the signs were more severe and peaked at 90 minutes post-dosing. No convulsions were reported at any dose, but catalepsy and decreased transfer arousal were seen in a few high dose animals. All animals had normal behavior by the next day and no additional adverse signs were recorded.

Cardiovascular effects: No directed cardiovascular safety studies were conducted. The sponsor assessed the potential for adverse cardiovascular effects in the repeated dose toxicology study in monkeys.

Effects of XP13512 on Action Potentials in Isolated Canine Cardiac Purkinje Fibers; Study #030522.DPW or RD2007/01531/00. This study was conducted by (b) (4) and was initiated on 6/12/03.

Three concentrations of test article were evaluated: 2, 20 or 200 µg/mL. No increases in APD_{60} or APD_{90} were found at any dose, but sotalol at 100µM elicited prolonged APD_{60} and APD_{90} without effect on resting membrane potential, action potential or maximal rate of depolarization.

Sponsor graphic:

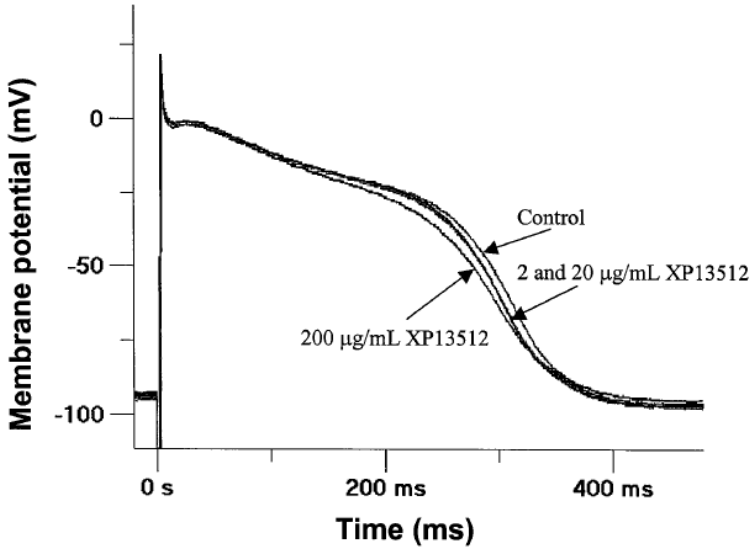


Figure 1. Effect of XP13512 on Cardiac Action Potentials.

Superimposed records before (control) and after equilibration (2, 20 and 200 µg/mL XP13512). Temperature = 37 ± 1° C, BCL = 2 s. Fiber ID: A 07 JK 030613 00.

Effects of XP13512 on Cloned hERG Channels Expressed in Mammalian Cells; Study #RD2007/01532/00XP011 or 030521.DPW. This study was conducted by (b) (4) and was initiated on 6/17/03. XP13512 was used as Lot #2892.A.03.2.

XP13512 was tested in HEK293 cells using standardized methodology. Terfenidine at 60 nM was the positive control, and inhibited the hERG current by 82.2%. DMSO served as the negative control.

Results: Neither 10 nor 100 µM XP13512 had a significant effect on hERG current.

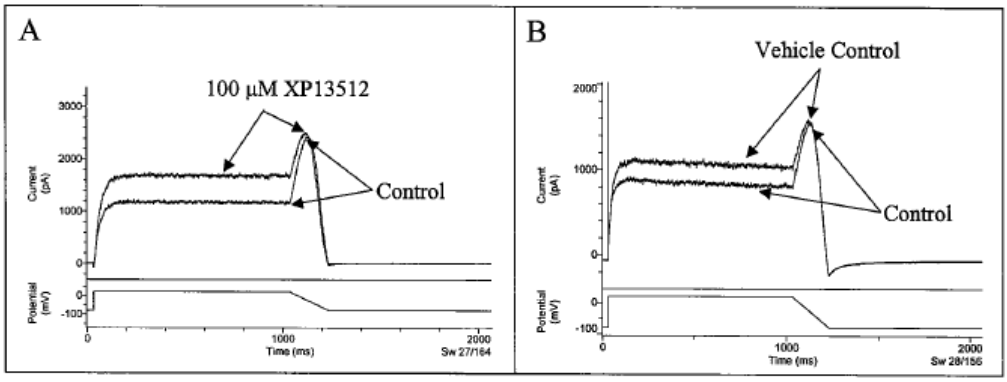


Figure 1: Sample hERG current traces before and during application of 100 µM XP13512 and the vehicle control.

Superimposed hERG currents [Current (pA); Time (ms)] were obtained using the voltage procedure described for measurement of the concentration-response relationship. The effects of 100 µM XP13512 and the vehicle control on hERG currents are shown in Panels A and B, respectively. The concentration-response voltage protocol is shown in the lower section of each panel.

Pulmonary effects:

A Pulmonary Safety Evaluation of XP13512 in the Rat; Study #1032-001. The GLP-compliant study was conducted by (b) (4)

In this study, 8 male rats were treated by oral gavage with 0 (0.5% methylcellulose), 75, 250 or 750 mg/kg XP13512 at 7.5 mL/kg. In this standardized study, the animals were monitored for pulmonary effects (respiratory rate, tidal and minute volumes) for an hour prior to dosing and continuously for 4 hrs post-dosing. Measurements were evaluated in 15 minute segments.

Results: No adverse effects on respiratory parameters were found in rats dosed up to 750 mg/kg with XP13512.

2.6.2.5 Pharmacodynamic drug interactions

No drug-drug interaction studies have been conducted using XP13512.

2.6.3 PHARMACOLOGY TABULATED SUMMARY

Table 1. Pharmacology **Overview** **Test Art**

Type of Study	Test System (Strain)	Method of Administration	Salt Form	GLP	Testing Facility	Report No. (Study No.)
Primary pharmacodynamic studies						
None[a]						
Secondary pharmacology studies						
Radioligand binding assay	Receptors, ion channels	In vitro	512	No	(b) (4)	RD2008/00262/00 (XP091)
Safety Pharmacology Studies						
Effects of general activity and behavior (Irwin study)	Rat (Wistar)	Oral (gavage)	512	Yes		RD2007/01533/00 (XP010)
Pulmonary safety evaluation	Rat (Wistar)	Oral (gavage)	512	Yes		RD2007/01530/00 (XP016)
hERG assay	HEK293 cells	In vitro	512	Yes		RD2007/01532/00 (XP011)
Effects on action potential	Isolated dog Purkinje fibers	In vitro	512	Yes		RD2007/01531/00 (XP012)

Key:
512 = XP13512.
HEK = Human embryonic kidney.
a. Although no primary pharmacology studies on XP13512 have been conducted, a discussion of the pharmacology of gabapentin is provided in m2.6.2, Section 2.1 based on information cited in the literature.

Testing Facility: (b) (4)

Table 2. Safety Pharmacology **Test Article:** XP13512

Organ Systems Evaluated	Species (Strain)	Method of Administration	Doses[a] (mg/kg)	No./ Group	Noteworthy Findings	GLP Compliant	Report No. (Study No.)
Overt central and peripheral effects	Rat (Wistar)	Oral (gavage)	75, 250, 750	5M/5F	No effects were noted at 75 mg/kg. The findings at 250 and 750 mg/kg included decreased body tone, apathy, ataxia and decreased touch response. Signs were observed within 30 minutes of dosing and completely reversed within 1 day.	Yes	RD2007/01533/00 (XP010)
Respiratory	Rat (Wistar)	Oral (gavage)	75, 250, 750	8M	No effects on respiratory function (respiration rate, tidal volume and minute volume) at any dose.	Yes	RD2007/01530/00 (XP016)
Effect on hERG tail current	HEK293 cells	In vitro	10, 100 µM	NA	No effects on hERG current.	Yes	RD2007/01532/00 (XP011)
Effect on action potential	Isolated dog Purkinje fibers	In vitro	2, 20, 200 µg/mL	NA	No effects on resting membrane potential, action potential amplitude, action potential maximum rate of rise or action potential duration at 60% or 90% repolarization (APD_{60} and APD_{90}).	Yes	RD2007/01531/00 (XP012)

Key:
HEK = Human embryonic kidney.
NA = Not applicable.
M = Male; F = Female.
a. Single dose administered.

2.6.4 PHARMACOKINETICS/TOXICOKINETICS

2.6.4.1 Brief summary

The ADME studies for XP13512 were single dose studies. This was considered a reasonable approach as XP13512 is a prodrug of gabapentin, which has been well investigated, e.g., in the published literature. In *in vitro* studies in human enterocytes, it appears that the prodrug is metabolized primarily by carboxylesterase 2 with a minor contribution by carboxylesterase 1. At all time points evaluated, the levels of gabapentin in plasma were ≤100x the levels of XP13512. The prodrug was detected in mice for ≤45 min post-dosing, in rats for ≤5 hrs post-dosing and ≤12 hrs in monkeys. Hydrolysis in the intestine is expected with oral dosing.

2.6.4.2 Methods of Analysis

Plasma and/or blood was evaluated for gabapentin, XP13512 and gabapentin lactam (a minor metabolite). Methodologies included protein precipitation followed by HPLC with LC-MS/MS. Samples were quenched as soon as possible with methanol to diminish the post-sampling conversion of the pro-drug to gabapentin after sample collection. The lower limits of quantitation were 0.02- 0.08 μg/mL in rat and monkey blood for gabapentin and 0.02 μg/mL for XP13512 and gabapentin lactam.

2.6.4.3 Absorption

Oral bioavailability of gabapentin following administration of XP13512 was ≤86.6% across the dose range (500- 5000 mg/kg) in rat single dose studies (RD2007/01519/00; RD2007/01699/00). After i.v. administration, XP13512 was converted to gabapentin with a $t_{½}$ of 5 minutes. When XP13512 was administered to monkeys at 174 mg/kg (capsular formulation), the bioavailability was ≤76% (RD2007/01476/00). When given as an oral gavage, the bioavailability was 50.5- 62.9%. This is in contrast to the oral bioavailability of gabapentin that decreases with increasing doses in rats, mice and monkeys.

Sponsor's tables:

Table 3.1. Pharmacokinetics of XP13512 and Gabapentin after Single Oral Administration of XP13512 to Mice, Rats and Monkeys

Species	Vehicle (Report No.)	Dose		No./ Group[b]	Mean Pharmacokinetic Parameters				
					Gabapentin			XP13512	
		mg/kg XP13512[a]	mg-eq GP/kg		C_{max} (µg/mL)	AUC_{0-inf} (µg.h/mL)	F[c] (%)	C_{max} (µg/mL)	AUC_{0-i} (µg.h/m
Mouse[d]	Solution in water (RD2007/01472/00)	192	100	4M	32.3	55.6	ND	0.08	ND
		962	500	4M	110	317	ND	1.68	1.99
		1923	1000	4M	231	618	ND	2.35	4.58
		3846	2000	4M	271	866	ND	4.83	5.65
Rat	Water[d] (RD2007/01471/00)	192	100	6M	22.5	102	64.9	0.19	0.50
		385	200	6M	34.1	190	60.6	0.18	0.95
		3846	2000	6M	155	2230	71.2	10.2	63.8
		4462	2320	7M	201	2073	57.0	13.3	56.9
	Suspension in 1% w/v methylcellulose/ 0.1% v/v Tween 80 in water (RD2007/01519/00)	500	260	3M/3F	107	353	86.6[e]	0.58	1.01
		2000	1040	3M/3F	157	1330	81.1[e]	0.90	5.16
		5000	2600	3M/3F	200	2450	70.3[e]	1.97	9.86
	Suspension in 0.5% w/v methylcellulose/ 0.1% v/v Tween 80 in water (RD2007/01699/00)	500	260	4M/4F	96.4	460	ND	0.601	1.49
		2000	1040	4M/4F	149	2160	ND	0.946	10.8
		5000	2600	4M/4F	189	2040	ND	1.68	11.9
	Suspension in 1% w/v methylcellulose/ 0.1% v/v Tween 80 in water (RD2007/01471/00)	4769	2480	6M	177	2228	57.5	11.6	59.6
Monkey	Hydroxypropyl methylcellulose capsules (RD2007/01476/00)	174	90.5	4M	60.3	392	76.3	2.03	4.79
		174	90.5	4M	62.9	356	69.0	1.97	6.31
	Suspension in 0.1% v/v Tween 80 in water (RD2007/01520/00)	500	260	2M/2F	90.3	791	51.1[e]	1.81	5.30
		2000	1040	2M/2F	281	3130	50.5[e]	17.7	100
	Suspension in 0.5% w/v methylcellulose/ 0.1% v/v Tween 80 in water (RD2007/01521/00)	250	130	3M/3F	78.4	551	71.1[e]	5.93	7.63
		750	391	3M/3F	143	1370	58.8[e]	7.55	17.1
		2000	1040	3M/3F	343	3900	62.9[e]	33.0	138

Key: GP = gabapentin; ND = Not determined; M= Male; F = Female.

a. 52% of molecular weight of XP13512 dose is gabapentin.

b. Number of animals per time point.

c. Bioavailability relative to intravenous gabapentin at 25 mg/kg in rats and 10 mg/kg in monkeys.

d. XP13497 (sodium salt) was administered.

e. Bioavailability determined, separate from cited report, based on mean systemic exposure values on Day 1 of repeat dose toxicity studies.

Table 3a. Pharmacokinetics: Absorption After a Single Dose

Test Article: XP13497 (sodium salt)
Location in CTD: m4.2.2.2
Report No.: RD2007/01472/00 **Study No.:** PK-2003-003

Species (strain): Mouse (CD-1)
Number of Animals/Gender (M/F): 28M
Feeding Condition: Fasted
Vehicle/Formulation: Water
Method of Administration: Oral (gavage)
Compound Administered: XP13497 (sodium salt)
Dose: 192, 962, 1923, 3846 mg/kg (100, 500, 1000, 2000 mg-eq GP/kg)
Sample: Plasma
Assay: Liquid chromatography with tandem mass spectroscopy (LC-MS/MS)

Samples quenched by low temperature prior to processing for plasma

Dose (mg-eq GP/kg):	100			500			1000			2000		
Analyte:	XP	GP	GL	XP	GP	GL	XP	GP	GL	XP	GP	GL
PK Parameters[a]:												
T_{max} (h)	0.75	0.25	0.25	0.25	0.50	0.5	0.25	0.25	0.25	1.0	0.50	0.5
C_{max} (µg/mL)	0.01	24.6	0.040	0.14	133	1.11	0.30	248	5.03	1.61	340	9.35
$AUC_{0\text{-}inf}$ (µg.h/mL)	ND	52.9	0.042	0.06	245	0.455	0.21	294	1.54	1.93	743	11.1
$t_{½}$ (h)	ND	1.0	0.6	0.6	1.8	0.3	1.4	1.0	0.6	1.8	0.7	0.4

Samples quenched by low temperature and the addition of an esterase inhibitor prior to processing for plasma

Dose (mg-eq GP/kg):	100			500			1000			2000		
Analyte:	XP	GP	GL	XP	GP	GL	XP	GP	GL	XP	GP	GL
PK Parameters[a]:												
T_{max} (h)	0.5	0.5	0.5	0.25	0.75	0.25	0.25	0.25	0.25	0.25	0.25	0.25
C_{max} (µg/mL)	0.08	32.3	0.032	1.68	110	0.237	2.35	231	0.343	4.83	271	0.509
$AUC_{0\text{-}inf}$ (µg.h/mL)	ND	55.6	0.027	1.99	317	0.386	4.58	618	0.750	5.65	866	ND
$t_{½}$ (h)	ND	1.6	0.6	2.1	1.5	1.1	2.9	2.2	2.5	1.2	2.1	ND

Key: XP = XP13512; GP = Gabapentin; GL = Gabapentin lactam; ND = Not determined.
a. Mean values (n=4/time point).

Table 3b. Pharmacokinetics: Absorption After a Single Dose

Test Article: XP13512, gabapentin hydrochloride, gabapentin lactam
Location in CTD: m4.2.2.2
Report No.: RD2007/01471/00 **Study No.:** PK-2003-004

Species (strain): Rat (Sprague Dawley)
Feeding Condition: Fasted
Method of Administration: Intravenous (bolus)
Sample: Blood or Plasma
Assay: Liquid chromatography with tandem mass spectroscopy (LC-MS/MS)

Compound Administered:	XP13512			Gabapentin hydrochloride		Gabapentin lactam
Number of Animals/Gender (M/F):	7M			6M		5M
Vehicle/Formulation:	0.1 M phosphate buffer, pH 7.4			Water		Water
Dose:	19 mg/kg (10 mg-eq GP/kg)			25 mg/kg		22 mg/kg
Analyte:	XP13512	Gabapentin	Gabapentin lactam	Gabapentin	Gabapentin lactam	Gabapentin lactam
PK Parameters:						
T_{max} (h)	-	0.08	0.5	-	0.5	-
C_{max} (µg/mL)	-	9.45	0.45	-	0.23	-
$AUC_{0\text{-}inf}$ (µg.h/mL)	1.75	15.6	1.51	39.2	1.31	67.3
$t_{½}$ (h)	0.09	1.2	2.5	1.8	5.9	2.9
CL (mL/h)	3260 (blood)	-	-	161 (plasma)	-	96.3 (plasma)
V_{ss} (mL)	91.1	-	-	345	-	210
MRT (h)	0.03	-	-	2.2	-	2.2
F (%)	-	99.2	-	-	-	-

Key: - = Not applicable.

Table 3e. Pharmacokinetics: Absorption After a Single Dose

Test Article: XP13512, gabapentin, gabapentin hydrochloride, XP13497 (sodium salt), XP16654 (calcium salt), XP17814 (S-enantiomer), XP17815 (R-enantiomer)
Location in CTD: m4.2.2.2
Report No.: RD2007/01478/00 **Study No.:** PK-2003-005

Species (strain): Rat (Sprague Dawley)
Feeding Condition: Fasted
Method of Administration: Intracolonic
Sample: Blood
Assay: Liquid chromatography with tandem mass spectroscopy (LC-MS/MS)

					Pharmacokinetic Parameters														
					Analyte:	Gabapentin					XP13512			Gabapentin lactam					
Compound Administered	Vehicle	No. of Males	Dose mg/kg	Dose mg-eq GP/kg	C_{max} (µg/mL)	T_{max} (h)	$t_{½}$ (h)	$AUC_{0\text{-}inf}$ (µg.h/mL)	F* (%)	C_{max} (µg/mL)	$t_{½}$ (h)	$AUC_{0\text{-}inf}$ (µg.h/mL)	C_{max} (µg/mL)	T_{max} (h)	$t_{½}$ (h)	$AUC_{0\text{-}inf}$ (µg.h/mL)	F* (%)
Gabapentin	C	7	25	-	0.55	0.5	1.5	1.3	3.38	-	-	-	0.02	1.5	2.7	0.09	0.16
Gabapentin hydrochloride	Water	6	25	-	0.26	3.2	2.2	1.86	4.75	-	-	-	0.02	3.4	5.2	0.29	0.48
XP13512	B	3	48	25	4.71	0.3	1.8	9.37	23.9	1.63	0.3	0.66	ND	ND	ND	ND	ND
	A	5	48	25	9.17	0.3	1.1	19.1	48.7	1.34	0.7	0.73	0.96	0.6	2.0	3.32	5.60
XP13497 (sodium salt)	Water	6	48	25	7.75	0.4	1.9	22.8	58.3	0.47	0.2	0.18	0.51	0.8	2.3	2.21	3.73
XP16654 (calcium salt)	B	5	48	25	7.95	0.5	1.4	17.7	45.2	0.70	0.5	0.25	0.72	0.9	3.0	2.87	4.84
XP17814 (S-enantiomer)	B	7	48	25	2.15	0.4	1.1	5.55	14.2	0.97	1.1	0.70	0.06	1.0	2.6	0.33	0.56
XP17815 (R-enantiomer)	B	6	48	25	1.82	0.4	1.8	6.58	16.8	0.24	0.2	0.09	0.30	0.4	3.0	1.24	2.09

Key: - = Not applicable; ND = Not determined; GP = Gabapentin; A = 1% methylcellulose and 0.1% Tween 80; B = Polyethylene glycol 400; C = Phosphate buffered saline.
* = Bioavailability based on comparison to data for intravenous gabapentin (see m2.6.5, Table 3b).

Table 3h. Pharmacokinetics: Absorption After a Single Dose

Test Article: XP13512, gabapentin, gabapentin hydrochloride
Location in CTD: m4.2.2.2
Report No.: RD2008/00464/00 **Study No.:** PK-2004-007

Species (strain): Monkey (cynomolgus)
Sample: Blood
Assay: Liquid chromatography with tandem mass spectroscopy (LC-MS/MS)

Method of Administration: **Intravenous (bolus)**

					Pharmacokinetic Parameters					
				Analyte:	Gabapentin					
Compound Administered	Vehicle	No. of Males	Dose (mg/kg)	Feeding Condition	C_0 (µg/mL)	$t_{½}$ (h)	$AUC_{0\text{-}inf}$ (µg.h/mL)	CL (mL/h)	V_z (mL)	MRT (h)
Gabapentin hydrochloride	Water	3	10	Fasted	21.9	3.43	47.5	211	1050	4.60

Method of Administration: **Oral**

						Pharmacokinetic Parameters							
					Analyte:	Gabapentin					XP13512		
Compound Administered	Formulation	No. of Males	Feeding Condition	Dose mg/kg	Dose mg-eq GP/kg	C_{max} (µg/mL)	T_{max} (h)	$t_{½}$ (h)	$AUC_{0\text{-}inf}$ (µg.h/mL)	F* (%)	C_{max} (µg/mL)	$t_{½}$ (h)	$AUC_{0\text{-}inf}$ (µg.h/mL)
Gabapentin	Neurontin capsules (3 x 100 mg)	4	Fasted	158	-	18.1	3.00	12.0	129	17.3	-	-	-
XP13512	Immediate release capsules (2 x 350 mg)	3	Fasted	352	183	88.6	4.67	4.46	626	72.1	1.47	1.64	5.61
	Sustained release tables (1 x 600 mg)	4	Fasted	294	153	57.9	6.00	5.07	528	72.6	0.977	3.43	4.61
		4	Fed	262	136	55.1	5.00	4.71	484	74.9	0.175	2.16	1.01

Key: - = Not applicable. GP = Gabapentin.
* = Bioavailability based on comparison to data for intravenous gabapentin in table above.

Pharmacokinetic parameters were assessed in the repeated dose and reproductive toxicity studies and the results were consistent with the single dose study results.

2.6.4.4 Distribution

The protein binding of XP13512 in human serum albumin was found to be relatively high (≤87%) over a concentration range of 5- 100 μM. Plasma protein binding of gabapentin is reportedly low (≤3%) in nonclinical models. Tissue distribution studies were conducted in albino (Sprague-Dawley) and pigmented (Long-Evans) rats using single oral gavage doses of 50 mg/kg. The highest concentrations were found at 2 hrs post-dosing in the pancreas and kidney. These findings are consistent with those reported for gabapentin after a single oral dose of 10 mg/kg. Radiolabeled material in pigmented skin and eyes declined more slowly than from plasma or other tissues with label <1 μg equivalent by 24 hrs post-dosing. Binding in pigmented rat and albino rat eyes was comparable at 6 hrs post-dosing (Mean values: 4.36 μg/g and 5.35 μg/g, respectively) and in pigmented and non-pigmented Long-Evans rat skin (6.51μg/g and 4.96 μg/g, respectively). After doses ≤385 mg/kg in rats, XP13497 was found in CSF only at the highest dose but the levels were <1/100th of the plasma concentrations at the same time point.

2.6.4.5 Metabolism

Four studies were conducted:
1) RD2007-01473 or PK-2003-002: **In vitro Metabolism of the Gabapentin Pro-drug XP13512 and its Sodium and Calcium Salts (XP13497 and XP16654)**

This study was conducted by XenoPort. Porcine pancreatin was used as a surrogate for secreted enzymes in the small intestine; cultured Caco-2 cells were used to assess stability in intestinal epithelial cells; rat and human plasma and rat and human liver cell homogenates were used to assess post-absorption metabolic stability. All analyses were done using LC/MS/MS methodologies.

Results: All test articles converted to gabapentin (36-43% converted within 1 hr) after incubation with porcine pancreatin. Caco-2 cells elicited gabapentin (75-87% within 1 hr). No lactam was produced in either assay. Stability in rat and human plasma was quite different with human plasma at ≤6% release of gabapentin and rat plasma with <55% release. Liver homogenates for all 3 entities showed similar releases of gabapentin, regardless of species of origin. No lactam was found after this incubation.

2) RD2007-01474 or PK-2003-015: **Analysis of Metabolites in Urine of Rats Following Oral Administration of ^{14}C- XP13512**

This non-GLP compliant study was conducted by XenoPort. Rat urine (N=3/sex) from the mass balance study (PK-R450-13512) was analyzed to determine the proportion of radioactivity due to parent compound (intact prodrug), gabapentin or other metabolites. The rats were dosed at 50 mg/kg of radiolabeled XP13512 and urine was collected for the first 24 hrs.

Results: No intact prodrug was detected in any of the samples but >99% of the radioactivity was due to gabapentin. Two metabolites (gabapentin-lactam [<0.4%] and a polar metabolite [<1.1%] were found. Total recovery was >95% primarily within the first 12 hrs post-dosing.

3) RD2008-00263 or XP095: **The Role of Human Carboxylesterases-1 and -2 (hCE-1 and hCE-2) in the Metabolism of XP13512**

This non-GLP compliant study was conducted by XenoPort. In this study, HEK 293 cells were used after verifying their specificity with clopidogrel and irinotecan (specific substrates) and microsomes expressing hCE-1 and hCE-2 were tested using p-nitrophenylacetate (non-specific substrate).

Results: A 4x increase in metabolism of XP13512 was found in hCE-1 expressing cells when compared to controls. In microsomes from cells expressing hCE-2, the metabolism was markedly increased (~20X) when compared to controls. Thus, hCE-2 has very specific activity towards XP13512 in vitro and is probably responsible for most of the metabolism within the human intestine.

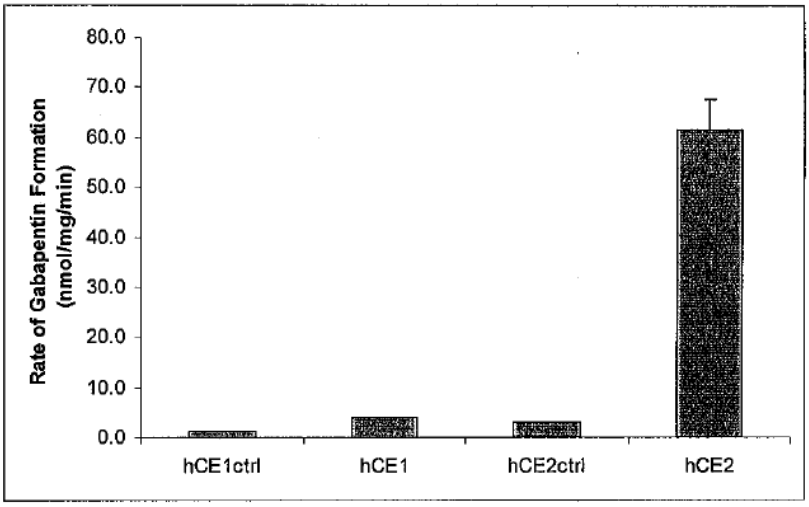
Sponsor table and graphic:

Table 5.3 Substrate Studies of XP13512 incubated with Expressed Human Carboxylesterase- 1 and -2 (hCE-1 and hCE-2)

Gabapentin Formation (nmol/mg/min)	Enzyme Preparation			
	hCE-1		hCE-2	
	Tetracycline-induced	Control Not induced	Tetracycline-induced	Control Not Induced
Individual Activity	3.9 ± 0.021	1.1 ± 0.057	61 ± 6.1	3.0 ± 0.12
Mean Specific Activity*	2.8	-	58	-

* Corrected for background hydrolysis in control

Figure 6.1 Rate of Gabapentin Formation from XP13512 incubated with Expressed Human Carboxylesterase 1 and 2 (hCE-1 and hCE-2) and Their Respective Controls



4) RD2008-00370 or XP092: **Evaluation of CYP450 Induction Potential by the Gabapentin Prodrug XP13512 and Gabapentin**

This non-GLP compliant study was conducted by XenoPort. Human hepatocytes induced with the pro-drug or gabapentin at clinically relevant doses were used to determine in vitro potential for CYP450 induction.

Results: No significant CYP450 induction was appreciated with XP13512 or gabapentin. An approximately 16x induction of CYP1A2 was demonstrated with the positive control agent (omeprazole at 20 μM) and of CYP2B6 (6.9x) and CYP3A4 (12x) for hepatocytes treated with rifampicin at 20 μM. Thus the positive controls performed as anticipated.

2.6.4.6 Excretion

Most of the radiolabeled XP13512 in rats was found in excreted urine within 12 hrs of dosing (RD2007/01487/00) with <1% recovered in feces.
Sponsor table:

Table 13a. Pharmacokinetics: Excretion

Test Article: XP13512
Location in CTD: m4.2.2.3
Report No.: RD2007/01487/00 **Study No.:** PK-2003-014

Species (Strain):	Rat (Sprague Dawley)
Gender (M/F)/Number of Animals:	3M/3F
Feeding Condition:	Fasted
Vehicle/Formulation:	0.1 M phosphate buffer
Method of Administration:	Oral (gavage)
Compound Administered:	XP13512
Duration of Dosing	Single
Dose (mg/kg):	50
Analyte:	^{14}C
Specific Activity:	0.044 µCi/mg
Assay:	Liquid scintillation counting (LSC)

Collection Time Point (h)	Mean Recovery (% of Radioactive Dose)						Mean Cumulative Recovery (% of Radioactive Dose)				
	Male			Female			Male			Female	
	Urine	Feces	Total	Urine	Feces	Total	Urine	Feces	Total	Urine	Feces
2	NC	NC	NC	NC	NC	NC	NC	NC	NC	NC	NC
6	53.2	NC	53.2	68.1	NC	68.1	70.2[a]	NC	70.2	68.2	NC
12	22.4	0.234	22.6	24.9	0.350	25.3	92.6	0.292	92.9	93.0	0.350
24	2.81	0.485	3.30	2.18	0.373	2.55	95.4	0.778	96.2	95.2	0.606
48	0.872	0.132	1.00	0.843	0.058	0.901	96.3	0.910	97.2	96.0	0.664
72	0.337	0.038	0.375	0.146	0.023	0.169	96.6	0.947	97.6	96.2	0.687
96	0.162	0.015	0.177	0.163	0.007	0.170	96.8	0.962	97.8	96.3	0.694
120	0.074	0.009	0.083	0.087	0.006	0.093	96.9	0.971	97.9	96.4	0.699
144	0.036	0.013	0.049	0.068	0.003	0.071	96.9	0.984	97.9	96.5	0.702
168	0.035	0.002	0.037	0.029	0.003	0.032	97.0	0.986	97.9	96.5	0.706
Subtotal	97.0	0.986	98.2[a]	96.5	0.706	97.9[b]	97.0	0.986	98.2[a]	96.5	0.706

Key: NC = Not calculated.
a. Includes data for single sample collected at 2 hours.
b. Includes that found in the cage washes.

2.6.4.7 Pharmacokinetic drug interactions

Not evaluated

2.6.4.8 Other Pharmacokinetic Studies

Report RD2007/01486/01 described the passive permeability in an artificial membrane assay. The permeability of XP13512 was pH-dependent while the permeability of gabapentin was not.

Transepithelial transport was assessed using Caco-2 and canine kidney cell monolayers in Study RD2007-01486. The results were consistent with an active uptake process. The transport across Caco-2 cells was not affected by the addition of verapamil (a P-glycoprotein inhibitor). XP13512 had a µM affinity for the sodium dependent multivitamin transporter (SMVT) in HEK cells that overexpressed SMVT. This transport was partially inhibited by the addition of excess biotin (a SMVT substrate) to the cultures.

2.6.4.9 Discussion and Conclusions

Pharmacokinetic studies were performed primarily via the oral route in mice, rats, rabbits and cynomolgus monkeys. Exposures to XP13512 were low in all species tested and

rapid hydrolysis to gabapentin was measured. Oral bioavailability to gabapentin was high in rats and monkeys in contrast to bioavailability after gabapentin administration where a dose-dependent decreased bioavailability was determined. In repeated dose studies in mice, rats and monkeys, C_{max} gabapentin values increased proportionally with dose in monkeys, but less than dose-proportionally in mice and rats. Mean AUC values for gabapentin after administration of XP13512 increased dose-proportionally in all species. No significant gender-related differences were appreciated.

XP13512 is moderately bound to human serum albumin. The test article appears to have a transport-mediated uptake in human Caco-2 cells. It dose not appear to be a substrate of human P-glycoprotein, but does appear to be a substrate for monocarboxylate transporter type-1 (MCT-1) and sodium dependent multivitamin transporter (SMVT) in vitro. The test article does not serve as a substrate or an inhibitor for human cytochrome P450 enzymes.

Distribution of radiolabeled XP13512 in albino and pigmented rats showed radioactivity at highest levels in the pancreas and kidney at 2 hrs post-dosing and essentially no radiolabel at 24 hrs post-dosing. The majority of the radiolabel was recovered in urine, primarily in the first 12 hrs.

2.6.4.10 Tables and figures to include comparative TK summary

2.6.5 PHARMACOKINETICS TABULATED SUMMARY

(Sponsor's tables)

Table 1. Pharmacokinetics — Overview — Test Article:)

Type of Study	Species (strain)	No./ Group	Method of Administration	Form	Dose (mg/kg/day)[a] or Concentration	Duration of Dosing (Sampling Occasions)	GLP	Testing Facility	Report No. (Study No.)	L (I
Absorption										
Pharmacokinetics	Mouse (CD-1)	28M	Oral (gavage)	497	192, 962, 1923, 3846	Single	No	XP	RD2007/01472/00 (PK-2003-003)	m
Toxicokinetics	Mouse (B6C3F1)	30M/30F	Oral (gavage)	512	500, 2000, 5000	7 days (Days 1 & 7)	Yes	(b) (4)	RD2007/01522/00 (XP023)	m
Toxicokinetics	Mouse (B6C3F1)	38M/38F	Oral (gavage)	512	500, 2000, 5000	13 weeks (Days 1 & 90)	Yes		RD2008/00324/00 (XP024)	m
Pharmacokinetics	Rat (SD)	6-7M	IV (bolus)	GP-HCl 512 GP-L	25 19 22	Single	No	XP	RD2007/01471/00 (PK-2003-004)	m
			Oral (gavage)	GP-HCl 497 512 654 814, 815	25, 50, 100, 200 192, 385, 3846, 4462 4769 48 48					
Pharmacokinetics	Rat (SD)	4-6M	IV (bolus) Intraperitoneal Oral (gavage)	512	48	Single	No	XP	RD2008/00212/00 (XP070)	m
Pharmacokinetics	Rat (SD)	3-7M	Intracolonic (bolus)	497, 654, 512, 814, 815	48	Single	No	XP	RD2007/01478/00 (PK-2003-005)	m
				GP, GP-HCl	25					
Toxicokinetics	Rat (Wistar)	5-6M/5-6F	Oral (gavage)	512	500, 2000, 5000	7 days (Days 1 & 7)	No	(b) (4)	RD2007/01519/00 (XP002)	m
Toxicokinetics	Rat (Wistar)	8M/8F	Oral (gavage)	512	500, 2000, 5000	2 weeks (Days 1 & 14)	Yes		RD2007/01699/00 (XP007)	m
Absorption (continued)										
Toxicokinetics	Rat (Wistar)	10M/10F	Oral (gavage)	512	500, 2000, 5000	2 weeks (Days 1 & 14)	Yes	(b) (4)	RD2007/01525/00 (XP038)	I
Toxicokinetics	Rat (Wistar)	10M/10F	Oral (gavage)	512	500, 2000, 5000	2 weeks (Days 1 & 14)	Yes		RD2007/01529/00 (XP049)	I
Toxicokinetics	Rat (Wistar)	15M/15F	Oral (gavage)	512	500, 2000, 5000	13 weeks (Days 1 & 90)	Yes		RD2007/01523/00 (XP025)	I
Toxicokinetics	Rat (Wistar)	12-18M/ 12-18F	Oral (gavage)	512	500, 2000, 5000	26 weeks (Days 1 & 175)	Yes		RD2007/01526/00 (XP046)	I
Toxicokinetics	Rat (SD)	6F	Oral (gavage)	512	200, 1000, 5000	11 days[b] (Days 1 & 11)	Yes	(b) (4)	RD2008/00144/00 (XP032)	m
Toxicokinetics	Rabbit (NZW)	3F	Oral (stomach tube)	512	200, 500, 1500, 5000	13 days[c] (Days 1 & 13)	Yes		RD2008/00145/00 (XP033)	m
Toxicokinetics	Rabbit (NZW)	3F	Oral (stomach tube)	512	200, 500, 2500	13 days[c] (Days 1 & 13)	Yes		RD2008/00146/00 (XP034)	m
Pharmacokinetics	Monkey (cynomolgus)	3-4M	IV (bolus)	GP-HCl	10	Single	No	XP	RD2007/01476/00 (PK-2003-007)	I
			Oral (gavage)	GP-HCl 497 654	10, 75 19, 144 144					
			Oral (capsules)	GP 512 497 654	81, 89 175 175, 177 173					
Pharmacokinetics	Monkey (cynomolgus)	3-4M	IV (bolus)	GP-HCl	10	Single	No	XP	RD2008/00464/00 (PK-2004-007)	I
			Oral (tablet)	GP, 512-SR 512	600 mg 700 mg					

Absorption (continued)

Pharmacokinetics	Monkey (cynomolgus)	3-4M	Oral (tablet)	512-SR	600 mg	Single	No	XP	RD2008/00219/00 (XP090)
Pharmacokinetics	Monkey (cynomolgus)	3-4M	Intracolonic (bolus)	GP-HCl 497, 512	10 19	Single	No	XP	RD2007/01475/00 (PK-2003-008)
Toxicokinetics	Monkey (cynomolgus)	1M/1F	Oral (gavage)	497	250, 500, 1000, 2000, 3500, 5000	Single	No	(b) (4)	RD2007/01520/00 (XP003)
				512	1000, 2000				
Toxicokinetics	Monkey (cynomolgus)	1M/1F	Oral (gavage)	512	2000, 5000	Single	Yes		RD2007/01698/00 (XP080)
Toxicokinetics	Monkey (cynomolgus)	2M/2F	Oral (gavage)	512	500, 2000	7 days (Days 1 & 8)	No		RD2007/01520/00 (XP003)
Toxicokinetics	Monkey (cynomolgus)	3M/3F	Oral (gavage)	512	250, 750, 2000	2 weeks (Days 1 & 14)	Yes		RD2007/01521/00 (XP008)
Toxicokinetics	Monkey (cynomolgus)	4M/4F	Oral (gavage)	512	500, 1000, 2000	13 weeks (Days 1 & 90)	Yes		RD2007/01524/00 (XP026)
Toxicokinetics	Monkey (cynomolgus)	6M/6F	Oral (gavage)	512	500, 1000, 2000	39 weeks (Days 1, 171 & 262)	Yes		RD2007/01528/00 (XP047)
Distribution									
Protein binding	Human serum albumin	NA	In vitro	512	5 to 100 µM	NA	No	XP	RD2007/01473/00 (PK-2003-002)
Transport across polarized cells	Caco-2 cells, MDCK cells	NA	In vitro	512, 497	100 to 200 µM	NA	No	XP	RD2007/01486/01 (BIO-2003-003)

Distribution (continued)

Transport across artificial lipid membranes	Artificial membranes	NA	In vitro	512, 497	50 µM	NA	No	XP	RD2007/01486/01 (BIO-2003-003)
Transport by intestinally-expressed transporters									
MCT-1	HEK cells	NA	In vitro	512, GP	Up to 10 mM	NA	No	XP	RD2007/01486/01 (BIO-2003-003)
MCT-1	Oocytes			512	0.25 to 1 mM				
SMVT	HEK cells			512, GP	Up to 1 mM				
SMVT	Oocytes			512	Up to 54 µM				
SMVT	HEK cells/TCN			512	Up to 100 µM				
LAT-1, OCTs	HEK cells			512, 497	NR				
Pgp substrate	Insect cell membranes			497	10 µM to 10 mM				
Pgp substrate	MDCK cells			512	20, 50, 100 µM				
Pgp inhibition	MES cells			512, 497	Up to 0.5 mM				
Transport by kidney transporters	Oocytes	NA	In vitro	[^{14}C]GP	>1 mM	NA	No	XP	RD2007/01486/01 (BIO-2003-003)
Quantitative tissue distribution	Rat (SD)	9M/9F	Oral (gavage)	[^{14}C]512	50	Single	Yes	(b) (4)	RD2007/01487/00 (PK-2003-014)
Quantitative tissue distribution	Rat (Long Evans)	24M	Oral (gavage)	[^{14}C]512	50	Single	No	XP	RD2008/00143/00 (XP-094)
CSF penetration	Rat	3-6M	Oral	GP-HCl	25, 100, 200	Single	No	XP	RD2007/01477/00

Metabolism									
Metabolic stability	Rat, human plasma/liver S9; Caco-2 cell S9 homogenate; Pancreatin	NA	In vitro	512, 497, 654, 814, 815	5 μM	NA	No	XP	RD2007/01473/00 (PK-2003-002)
Metabolic stability	Rat, dog, monkey, human plasma & intestinal, lung, liver, kidney S9	NA	In vitro	497	10 μM	NA	No	XP	RD2007/01473/00 (PK-2003-002)
Role of carboxylesterases	Human	NA	In vitro	512	50 μM	NA	No	XP	RD2008/00263/00 (XP095)
Metabolite analysis (urine)	Rat (SD)	3M/3F	Oral (gavage)	[^{14}C]512	50	Single	No	XP	RD2007/01474/00 (PK-2003-015)
CYP substrate (liver S9)	Human	NA	In vitro	512	5 μM	NA	No	XP	RD2007/01473/00 (PK-2003-002)
CYP induction (hepatocytes)	Human	NA	In vitro	512 GP	0.2, 2, 20 μM 10, 100, 1000 μM	NA	No	XP	RD2008/00370/00 (XP092)
CYP inhibition	Bacculosomes	NA	In vitro	512	Up to 400 μM	NA	No	XP	RD2007/01473/00 (PK-2003-002)
Excretion									
Mass Balance	Rat (SD)	3M/3F	Oral (gavage)	[^{14}C]512	50	Single	Yes	(b) (4)	RD2007/01487/00 (PK-2003-014) m4.2.2.3

Key:

512 = XP13512.
512-SR = XP13512 Extended Release.
497 = XP13512 sodium salt.
654 = XP13512 calcium salt.
814 = XP13512 S-enantiomer.
815 = XP13512 R-enantiomer.
[^{14}C]512 = ^{14}C-XP13512.
GP = Gabapentin.
GP-HCl = Gabapentin hydrochloride.
GP-L = Gabapentin lactam.
[^{14}C]GP = ^{14}C-gabapentin.
M = Male; F = Female.
IV = Intravenous.
Caco-2 = Human colon cancer cell line-2.
CYP = Cytochrome P450.
HEK = Human embryonic kidney.
LAT-1 = Large amino acid transporter.
MES = MES-SA-DX5.
MDCK = Madin-Darby canine kidney.
NA = Not applicable.
NR = Not reported.
NZW = New Zealand white.
OCT2 = Organic cation transporter.
Pgp = P-glycoprotein.
SD = Sprague Dawley.
TCN = Tetracycline.

Testing Facility:
(b) (4)
XP = Xenoport Inc.

a. In some reports (e.g., primarily single dose pharmacokinetics), the dose is quoted in terms of mg-eq gabapentin/kg. For a dose of XP13512, gabapentin was equivalent to ~52% of the molecular weight of XP13512 (i.e. 100 mg-eq GP/kg is approximately equivalent to 192 mg/kg XP13512).
b. Days 7 through 17 post coitum.
c. Days 7 through 19 post coitum.

2.6.6 TOXICOLOGY

2.6.6.1 Overall toxicology summary

General toxicology:

Repeated dose testing via the oral route was performed in several species: up to 26 weeks in albino rats at doses up to 5000 mg/kg/d, up to 39 weeks in cynomolgus monkeys at doses up to 2000 mg/kg/d. In rats, the doses were 0, 500, 2000 or 5000 mg/kg/d. As in the previous, shorter term rat studies, increased age-related chronic progressive nephropathy with hyaline droplet formation was noted in all treated male groups. Reversal was incomplete at the end of the recovery period. Centrilobular hepatocellular hypertrophy was described in the high dose animals, but was reversed by the end of the 1 month recovery period. No NOAEL for the histologic renal findings was found in this study but clinical chemistries and urinalyses were not affected. Cynomolgus monkeys were treated by oral gavage with 0, 250, 1000 or 2000 mg/kg/d of XP13512. No adverse effects of treatment were found in any of the parameters evaluated. The NOEL is determined to be 2000 mg/kg/d. Exposures to gabapentin at the highest dose were 3370 µg.h/mL at the end of the 9 month period while exposures to XP13512 were 54.3 µg.h/mL at the same time point demonstrating extensive hydrolysis of the test article to gabapentin. The associated Cmax values were 366 µg/mL and 13.7 µg/mL, respectively.

Genetic toxicology:

XP13512 was not genotoxic in multiple Ames assays, the micronucleus or the UDS assays. However, it was positive in the chromosomal aberration assay in human lymphocytes. The etiology of this finding was proposed to be the release of acetaldehyde during the (b) (4) (b) (4) potential (b) (4) impurities were found to be genotoxic in the Ames assays, but the levels in the final product are below the level of concern.

Carcinogenicity:

In the mouse 2-year carcinogenicity study, no increases in any tumor type were detected. In mice treated with 0, 500, 2000 or 5000 mg/kg/d by oral gavage, XP13512 treatment caused decreased survival in the mid and high dose males and increased body weights in the high dose animals. No other significant findings were appreciated in treated animals except for a modest exacerbation of age-related axonal/myelin degeneration of the sciatic nerve in the females at 2000 mg/kg/d and both sexes at 5000 mg/kg/d.

In the rat carcinogenicity study, Wistar rats were treated for up to 104 weeks with 0, 500, 2000 or 5000 mg/kg/d of XP13512 in Tween 80 and methylcellulose by oral gavage. The 2000 and 5000 mg/kg/d males were terminated early (Weeks 97 and 90, respectively) due to exacerbation of chronic progressive nephropathy.

Females were not similarly affected. There was an increased incidence of pancreatic acinar cell hyperplasia, adenomas and carcinomas in both sexes at 5000 mg/kg/d and in males at 2000 mg/kg/d. The decreased survival and early termination in the 2000 and 5000 mg/kg/d males may be responsible for a lesser incidence of both non-neoplastic and neoplastic lesions. Thus, XP13512 is considered a carcinogen in rats under the conditions of this study.

Reproductive toxicology:

A complete battery of reproductive toxicity testing was conducted in rats and rabbits and no adverse effects were found on fertility or embryofetal development parameters. Maternal toxicity was shown by adverse clinical signs, decreased body weights and premature parturition (rabbits only) was evident in all studies. Embryo-fetal toxicity was found in rat pups at 5000 mg/kg/d and rabbit kits at 2500 mg/kg/d.

Special toxicology:

XP13512 was not shown to be a skin sensitizer and it was a non-irritant on skin. It was found to be an ocular irritant.

2.6.6.2 Single-dose toxicity

Studies were not reviewed as the MTD/MFD was utilized in the repeat-dose toxicity studies and no additional toxicities were described in the single dose studies.

2.6.6.3 Repeat-dose toxicity

Study title: **2-Week Oral Gavage Toxicity and Toxicokinetic Study with XP13512 in Cynomolgus Monkeys**

Key study findings: Although significant vomition was seen in the 2000 mg/kg/d females on 7 of 14 days of the study, the sponsor did not consider this to be an adverse effect and stated the NOAEL to be >2000 mg/kg/d in cynomolgus monkeys. No other adverse effects were reported.

Study no.: RD2007/01521/00 or 7401-111 or XP008
Volume and page #: Electronic submission
Conducting laboratory and location: (b) (4)
Date of study initiation: 6/3/03
GLP compliance: Yes
QA report: yes
Drug, lot #, and % purity: Lot 2892.A.03.1 at 98.4% purity and Lot 2892.A.03.2 at 97.3% purity

Methods

Doses: 0 (vehicle), 250, 750 or 2000 mg/kg/d
Species/strain: Cynomolgus monkeys

Number/sex/group or time point (main study): 3
Route, formulation, volume, and infusion rate: Oral gavage in 0.1% Tween 80+ 0.5% methylcellulose at 10 mL/kg
Satellite groups used for toxicokinetics or recovery: None
Age: 2-3 years of age
Weight: 2.3- 2.8 kg
Sampling times: Days 1 and 14 at 0 and 1 hr post-dosing

Observations and times:
Mortality and clinical signs: Twice daily
Body weights: Prior to dosing and weekly
Food consumption: Daily
Ophthalmoscopy: Prior to dosing and Day 11
EKG: Prior to dosing and at 0 and 4 hrs post-dosing during Week 2 under ketamine anesthesia
Hematology: Prior to dosing and Day 14
Clinical chemistry: As for hematology
Urinalysis: As for hematology
Gross pathology: All animals on study
Organ weights: Adrenals, brain, epididymides, heart, kidney, liver with drained gallbladder, lung, ovary, pituitary, prostate, salivary gland, seminal vesicles, spleen, testes, thyroid/parathyroids, uterus
Histopathology: Adequate Battery: yes
Peer review: Not specified

Results
Mortality: There were no premature decedents.

Clinical signs: Vomition was the primary adverse effect in females dosed at 2000 mg/kg/d (noted for 7 of 14 days). The sponsor did not consider this to be adverse as it was "not frequently observed".

Body weights: Weights were comparable across dose groups and controls.

Food consumption: No adverse effects were reported on consumption.

Ophthalmoscopy: No treatment-engendered lesions were observed.

EKG: All tracings were within normal limits.

Hematology: No significant intergroup differences were conveyed in the study report.

Clinical chemistry: No significant test article-related adverse effects were determined.

Urinalysis: No test article-related effects were discerned.

Gross pathology: No consistent lesions were described.

Organ weights: No treatment-related adverse effects on organ weights were found.

Histopathology: Adequate Battery: yes
Peer review: Not specified
No consistent adverse, treatment-related lesions were appreciated.

Toxicokinetics: At essentially all sampling times, the levels of gabapentin were very high when compared to the prodrug levels. Concentrations of gabapentin reached Cmax within 1.5-8 hrs post-dosing and declined thereafter. Gabapentin exposure increased with increasing dose, but the absorption may have been delayed at the higher doses as shown by the AUC of gabapentin having a more dose-proportional relationship than Cmax. No gender differences were appreciated and no accumulation was evidenced.

Sponsor table:

Table 4.1 Toxicokinetic Data for Gabapentin in Blood After Oral Administration of XP13512 to Monkeys in Study XP008

Group	Dose (mg/kg)	Day	Gender	Animal	T1/2 (hr)	T_{max} (hr)	C_{max} (µg/mL)	T_{last} (hr)	C_{last} (µg/mL)	AUC_{last} (µg.hr/mL)	AUC_{all} (µg.hr/mL)	$AUC_{(0\text{-}inf)}$ (µg.hr/mL)
2	250	1	Male	I00331	3.53	1.50	62.5	24	1.09	480	480	486
				109946	3.52	1.50	61.9	24	0.814	405	405	409
				109973	4.17	4.00	87.8	24	2.56	767	767	782
				MEAN	3.74	2.33	70.7	24	1.49	551	551	559
				SD	0.37	1.44	14.8	0	0.938	191	191	197
			Female	I00110	4.14	1.50	73.7	24	1.97	574	574	586
				I00127	3.95	2.00	64.5	24	1.37	411	411	419
				I00129	3.33	2.00	120	24	0.768	622	622	625
				MEAN	3.81	1.83	86.2	24	1.37	536	536	543
				SD	0.43	0.29	29.9	0	0.599	110	110	110
			Combined	MEAN	3.77	2.08	78.4	24	1.43	543	543	551
				SD	0.36	0.97	22.7	0	0.707	140	140	143
2	250	14	Male	I00331	3.78	1.50	130	24	1.02	646	646	652
				109946	3.82	1.50	78.4	24	0.716	434	434	438
				109973	4.16	2.00	141	24	2.74	932	932	948
				MEAN	3.92	1.67	117	24	1.49	671	671	680
				SD	0.21	0.29	33.5	0	1.09	250	250	256
			Female	I00110	4.19	2.00	111	24	1.85	693	693	704
				I00127	5.15	1.50	104	24	2.02	567	567	582
				I00129	3.75	1.50	137	24	1.43	800	800	807
				MEAN	4.36	1.67	118	24	1.77	687	687	698
				SD	0.71	0.29	17.4	0	0.304	116	116	113
			Combined	MEAN	4.14	1.67	117	24	1.63	679	679	689
				SD	0.53	0.26	23.9	0	0.731	174	174	177

Displayed values were rounded to three significant figures after calculation of mean values and ratios.

Table 4.1 (continued) Toxicokinetic Data for Gabapentin in Blood After Oral Administration of XP13512 to Monkeys in Study XP008

Group	Dose (mg/kg)	Day	Gender	Animal	T1/2 (hr)	T_{max} (hr)	C_{max} (µg/mL)	T_{last} (hr)	C_{last} (µg/mL)	AUC_{last} (µg.hr/mL)	AUC_{all} (µg.hr/mL)	$AUC_{(0\text{-}inf)}$ (µg.hr/mL)
3	750	1	Male	I00001	4.82	2.00	119	24	9.40	1450	1450	1520
				I00334	3.68	2.00	184	24	3.56	1350	1350	1370
				I00361	4.08	4.00	140	24	5.03	1290	1290	1320
				MEAN	4.19	2.67	147	24	6.00	1370	1370	1400
				SD	0.58	1.15	33.0	0	3.04	81	81	102
			Female	I00062	3.69	2.00	157	24	4.46	1500	1500	1520
				I00064	3.54	6.00	118	24	3.49	1140	1140	1160
				I00394	3.53	4.00	143	24	3.04	1330	1330	1340
				MEAN	3.59	4.00	139	24	3.66	1320	1320	1340
				SD	0.09	2.00	19.7	0	0.723	180	180	183
			Combined	MEAN	3.89	3.33	143	24	4.83	1340	1340	1370
				SD	0.50	1.63	24.7	0	2.35	127	127	137
3	750	14	Male	I00001	5.56	2.00	185	24	11.8	1780	1780	1870
				I00334	4.58	1.50	148	24	3.21	916	916	937
				I00361	4.08	4.00	173	24	4.44	1440	1440	1470
				MEAN	4.74	2.50	169	24	6.48	1380	1380	1430
				SD	0.76	1.32	18.9	0	4.64	434	434	468
			Female	I00062	5.01	2.00	227	24	7.52	1620	1620	1680
				I00064	3.71	2.00	210	24	3.92	1700	1700	1720
				I00394	3.97	4.00	157	24	4.24	1330	1330	1360
				MEAN	4.23	2.67	198	24	5.23	1550	1550	1590
				SD	0.69	1.15	36.3	0	2.00	195	195	200
			Combined	MEAN	4.48	2.58	183	24	5.85	1470	1470	1510
				SD	0.71	1.11	30.3	0	3.27	315	315	334

Displayed values were rounded to three significant figures after calculation of mean values and ratios.

Table 4.1 (continued) Toxicokinetic Data for Gabapentin in Blood After Oral Administration of XP13512 to Monkeys in Study XP008

Group	Dose (mg/kg)	Day	Gender	Animal	T1/2 (hr)	T_{max} (hr)	C_{max} (µg/mL)	T_{last} (hr)	C_{last} (µg/mL)	AUC_{last} (µg.hr/mL)	AUC_{all} (µg.hr/mL)	$AUC_{(0-inf)}$ (µg.hr/mL)
4	2000	1	Male	I00008	6.57	4.00	372	24	38.0	3630	3630	3990
				I00323	4.84	6.00	439	24	28.3	4250	4250	4450
				I00350	5.07	6.00	382	24	35.9	4390	4390	4650
				MEAN	5.50	5.33	398	24	34.1	4090	4090	4360
				SD	0.94	1.15	36.3	0	5.09	407	407	342
			Female	I00114	3.38	8.00	202	24	7.80	2370	2370	2410
				I00388	3.91	2.00	306	24	13.3	3370	3370	3450
				I00398	6.63	4.00	359	24	41.4	4080	4080	4480
				MEAN	4.64	4.67	289	24	20.8	3280	3280	3450
				SD	1.75	3.06	80.2	0	18.0	859	859	1030
			Combined	MEAN	5.07	5.00	343	24	27.4	3680	3680	3900
				SD	1.34	2.10	81.7	0	13.9	748	748	852
4	2000	14	Male	I00008	4.65	4.00	525	24	21.8	4580	4580	4720
				I00323	4.91	4.00	515	24	22.8	4600	4600	4770
				I00350	5.76	4.00	402	24	34.1	4720	4720	5000
				MEAN	5.11	4.00	481	24	26.3	4630	4630	4830
				SD	0.58	0.00	68.4	0	6.82	74	74	149
			Female	I00114	4.00	4.00	456	24	14.5	4160	4160	4240
				I00388	6.43	2.00	400	24	24.8	3340	3340	3570
				I00398	5.09	4.00	374	24	22.6	3740	3740	3910
				MEAN	5.17	3.33	410	24	20.6	3750	3750	3910
				SD	1.22	1.15	41.9	0	5.44	411	411	338
			Combined	MEAN	5.14	3.67	445	24	23.4	4190	4190	4370
				SD	0.86	0.82	63.9	0	6.32	553	553	557

Displayed values were rounded to three significant figures after calculation of mean values and ratios.

Study title: **2-Week Oral Toxicity Study of XP13512 in Rats**

Key study findings: No NOAEL was determined for this 2-week oral toxicity study in rats at doses of 500, 2000 or 5000 mg/kg/d on the basis of hypoactivity in all treated animals and increased hyaline droplet formation in the renal epithelium of treated males at all doses. No other significant findings were appreciated in any of the other parameters evaluated. This study was neither an MTD nor an MFD study but 5000 mg/kg/d is generally acceptable as the maximal dose to be administered in toxicology studies.

Study no.: 1032-015 or RD2007/01525/00XP038
Volume and page #: Electronic submission
Conducting laboratory and location: (b) (4)
Date of study initiation: 6/29/04
GLP compliance: Yes
QA report: yes
Drug, lot #, and % purity: Batch 3 at 99.5% purity

Methods

Doses: 0 (vehicle), 500, 2000 or 5000 mg/kg/d
Species/strain: Wistar [Crl: (WI)BR] rats
Number/sex/group or time point (main study): 10

Route, formulation, volume, and infusion rate: Oral gavage in 0.1% Tween 80® + 0.5% methylcellulose at 20 mL/kg

Satellite groups used for toxicokinetics or recovery: Not applicable

Age: 7 weeks at study initiation

Weight: 165- 295 gms

Sampling times: TK samples taken on Days 1 and 14 at pre-dose and 1 hr post-dosing.

Observations and times:

Mortality and clinical signs: Twice daily
Body weights: Weekly
Food consumption: Weekly
Ophthalmoscopy: Prior to study initiation and prior to necropsy
EKG: Not performed
Hematology: At study termination
Clinical chemistry: At study termination
Urinalysis: At study termination
Gross pathology: All animals on study
Organ weights: Brain, adrenals, epididymides, heart, kidneys, liver, lung, pituitary, prostate, salivary gland, seminal vesicles, spleen, testes, thymus, thyroid/parathyroids, ovaries, uterus
Histopathology: Adequate Battery: yes for control and high dose only; liver, kidney, pancreas and gross lesions from other groups
Peer review: Not specified

Results

Mortality: There were several incidental deaths: 1 female at 500 mg/kg/d (#1058); post-bleeding "accident"), 1/sex at 2000 mg/kg/d (#1022 and 1064; causes of death undetermined) and 1 male (#11035; gavage accident) at 5000 mg/kg/d.

Clinical signs: A significant dose-related hypoactivity was observed in all treated animals early in the study (Days 1-3) with recovery by approximately 6 hrs post-dosing. Decreased activity was noted at weekly observation times in the 2000 and 5000 mg/kg/d males.

Body weights: No consistent adverse effects of treatment were noted.

Food consumption: Consumption was comparable across groups over the 2 week dosing period.

Ophthalmoscopy: No treatment-engendered lesions were described.

Hematology: The high dose animals showed mild decreases in red cell parameters (erythrocyte count, hemoglobin, hematocrit) with increased reticulocyte counts.

Clinical chemistry: Cholesterol levels were slightly-moderately increased in the mid and high dose groups.

Urinalysis: No intergroup differences were found.

Gross pathology: No significant treatment-associated gross lesions were detected.

Organ weights: Weights of livers ($p<0.01$; ~22%) and spleens ($p<0.01$; ~30%) were increased in the high dose females, but no histologic correlates were determined. Therefore, the biological significance of this finding is uncertain.

Histopathology: Increased hyaline droplets were found in the renal tubular epithelium of treated males and were described as minimal to moderate in severity. The incidence and severity increased with increasing dose (0, 5 (minimal), 2 (minimal)/7 (mild)/1 (moderate), 4 (mild)/6 (moderate) for the respective dose groups). This finding may be associated with the ά2 μ-globulin found in male rats.

Toxicokinetics: XP13512 was rapidly absorbed and converted to gabapentin. Exposures to gabapentin increased less than dose proportionally with doses up to 2000 mg/kg/d, but no further increase was noted at the 5000 mg/kg/d dose. Exposures to XP13512 were low at all doses and <1% of the associated gabapentin dose at both time points at all doses.

Sponsor tables:

Table 4.3 Mean Toxicokinetic Parameters for XP13512 in Blood of Rats After Oral Dosing of XP13512 – DAY 1

Group	Dose	N	Gender	$C_{0\ hr}$ (μg/mL)	$C_{1\ hr}$ (μg/mL)
2	500 mg/kg/day	5	Male	0	0.360
		5	Female	0	0.521
		10	Combined Ave.	0	0.441
3	2000 mg/kg/day	5	Male	0	1.26
		4	Female	0	1.07
		9	Combined Ave.	0	1.18
4	5000 mg/kg/day	5	Male	0	0.928
		5	Female	0	0.957
		10	Combined Ave.	0	0.942

Table 4.1 Mean Toxicokinetic Parameters for Gabapentin in Blood of Rats After Oral Dosing of XP13512 – DAY 1

Group	Dose	N	Gender	$C_{0\ hr}$ (μg/mL)	$C_{1\ hr}$ (μg/mL)
2	500 mg/kg/day	5	Male	0	87.9
		5	Female	0	79.4
		10	Combined Ave.	0	83.7
3	2000 mg/kg/day	5	Male	0	176
		4	Female	0	110
		9	Combined Ave.	0	146
4	5000 mg/kg/day	5	Male	0	139
		5	Female	0	149
		10	Combined Ave.	0	144

Table 4.4 Mean Toxicokinetic Parameters for XP13512 in Blood of Rats After Oral Dosing of XP13512 – DAY 14

Group	Dose	N	Gender	$C_{0\ hr}$ (µg/mL)	$C_{1\ hr}$ (µg/mL)
2	500 mg/kg/day	5	Male	0.048	0.380
		5	Female	0.018	0.282*
		10	Combined Ave.	0.033	0.336
3	2000 mg/kg/day	5	Male	0.016	1.82
		4	Female	0.026	1.73
		9	Combined Ave.	0.021	1.77
4	5000 mg/kg/day	5	Male	0.014	1.62
		5	Female	0.005	1.76
		10	Combined Ave.	0.010	1.69

*Data are mean for 4 animals.

Table 4.2 Mean Toxicokinetic Parameters for Gabapentin in Blood of Rats After Oral Dosing of XP13512 – DAY 14

Group	Dose	N	Gender	$C_{0\ hr}$ (µg/mL)	$C_{1\ hr}$ (µg/mL)
2	500 mg/kg/day	5	Male	0.292	95.3
		5	Female	0.603	97.3*
		10	Combined Ave.	0.447	96.2
3	2000 mg/kg/day	5	Male	5.77	207
		4	Female	4.04	176
		9	Combined Ave.	4.90	192
4	5000 mg/kg/day	5	Male	17.7	266
		5	Female	10.8	265
		10	Combined Ave.	14.2	265

*Data are mean for 4 animals.

Study title: **2-Week Oral Toxicity Study of XP13512 in Rats**

Key study findings: Wistar rats were treated by oral gavage with 0, 500, 2000 or 5000 mg/kg/d XP13512 for 14 days. Hypo- and/or hyper-activity were noted in all dose groups. As in other rat studies, increased hyaline droplet formation was appreciated in the renal tubular epithelium of the mid and high dose males.

Study no.: 1032-038 or RD2007/01529/00XP049
Volume and page #: Electronic submission
Conducting laboratory and location: (b) (4)
Date of study initiation: 12/30/04
GLP compliance: Yes
QA report: yes
Drug, lot #, and % purity: Batch 7 at

Methods

Doses: 0 (vehicle), 500, 2000 or 5000 mg/kg/d
Species/strain: Wistar [Crl: (WI)BR] rats
Number/sex/group or time point (main study): 10
Route, formulation, volume, and infusion rate: Oral gavage in 0.1% Tween 80® + 0.5% methylcellulose at 20 mL/kg

Satellite groups used for toxicokinetics or recovery:
Age: 7 weeks of age at study initiation
Weight: 150- 220 gms
Sampling times: TK samples were taken from 5/sex/group/time point on Days 1 and 14 at predose and 1 hrs post-dosing

Observations and times:
Mortality: Twice daily
Clinical signs: Twice daily
Body weights: Weekly
Food consumption: Weekly
Ophthalmoscopy: Prior to study initiation and prior to necropsy
EKG: Not performed
Hematology: At necropsy
Clinical chemistry: At necropsy
Urinalysis: At necropsy
Gross pathology: All study animals
Organ weights: Adrenals, brain, epididymides, ovary, testes, heart, kidney, liver, lung, pituitary, prostate, salivary gland, seminal vesicles, spleen, thymus, thyroid/parathyroids, uterus
Histopathology: Adequate Battery: yes for control and high dose only with from remaining groups
Peer review: Not specified

Results
Mortality: There were no premature decedents.

Clinical signs: Both hypo- and hyper-activity were noted in the low and mid dose groups but only hypoactivity was observed at the 5000 mg/kg/d dose.

Body weights: No adverse effects of dosing were measured.

Food consumption: No effects of treatment were reported on feed consumption.

Ophthalmoscopy: No treatment-engendered lesions were described.

Hematology: As in other rat studies, slightly decreased red cell parameters were seen at the 5000 mg/kg/d dose.

Clinical chemistry: Slightly increased cholesterol levels were recorded for the mid and high dose animals, but this finding is not considered biologically significant in the rodent at this degree of severity.

Urinalysis: No adverse effects of treatment were determined for urinary parameters.

Gross pathology: There were no treatment-related gross lesions discussed.

Organ weights: Liver weights were increased in the high dose animals (≤17%), but no histologic correlates were observed. This increase is not considered biologically meaningful.

Histopathology: Adequate Battery: yes, for control and high dose animals
Peer review: Not specified

As in other rat studies, increased hyaline droplet formation was appreciated in the renal tubular epithelium in the 2000 and 5000 mg/kg/d males. Although the incidence increased with increasing dose, the severity did not exceed mild in any animal. The sponsor suggested that the droplets were composed of the xenobiotic (test article) or its metabolite bound to alpha-2μ-globulin. This is a reasonable conclusion, although the mechanism was not definitively explored.

Sponsor table:

Summary of Microscopic Observations - MALE

Table 9 Terminal

Tissue / Observation	Severity	0 mg/kg/day (Control)	500 mg/kg/day	2000 mg/kg/day	5000 mg/kg/day
Number of Animals Examined		10	10	10	10
eyes		(10)	(0)	(0)	(10)
within normal limits		10	0	0	10
eyes, optic nerves		(10)	(0)	(0)	(10)
within normal limits		10	0	0	10
harderian glands		(10)	(0)	(0)	(10)
within normal limits		10	0	0	10
heart		(10)	(0)	(0)	(10)
within normal limits		10	0	0	10
kidneys		(10)	(10)	(10)	(10)
cyst	- minimal	1	0	0	0
hyaline, droplets, increased		0	0	5	9
	- minimal	0	0	3	5
	- mild	0	0	2	4
hydronephrosis, unilateral		1	0	1	0
	- minimal	0	0	1	0
	- mild	1	0	0	0
regeneration, tubular	- minimal	3	1	1	6
within normal limits		6	9	3	0
lacrimal glands, exorbital		(10)	(0)	(0)	(10)
within normal limits		10	0	0	10

( ) - Number observed

Toxicokinetics: Effective hydrolysis of XP13512 to gabapentin was seen at all doses and exposure to gabapentin increased with increasing dose in a less than dose-proportional manner. Little evidence of accumulation was found.

Sponsor tables:

Table 4.1 Mean Concentrations of Gabapentin in Blood of Rats After Oral Dosing of XP13512 – DAY 1

Group	Dose	N	Gender	$C_{0\ hr}$ (µg/mL)	$C_{1\ hr}$ (µg/mL)
2	500 mg/kg/day	5	M	0	64.5
		5	F	0	59.0
		10	Combined Ave.	0	61.8
3	2000 mg/kg/day	5	M	0	138
		5	F	0	137
		10	Combined Ave.	0	137
4	5000 mg/kg/day	5	M	0	174
		5	F	0	129
		10	Combined Ave.	0	152

Table 4.2 Mean Concentrations of Gabapentin in Blood of Rats After Oral Dosing of XP13512 – DAY 14

Group	Dose	N	Gender	$C_{0\ hr}$ (µg/mL)	$C_{1\ hr}$ (µg/mL)
2	500 mg/kg/day	5	M	0.0936	75.9
		5	F	0.0552	83.8
		10	Combined Ave.	0.0744	79.8
3	2000 mg/kg/day	5	M	0.367	151
		5	F	0.462	181
		10	Combined Ave.	0.415	166
4	5000 mg/kg/day	5	M	2.24	173
		5	F	1.19	300
		10	Combined Ave.	1.71	236

Table 4.3 Mean Concentrations of XP13512 in Blood of Rats After Oral Dosing of XP13512 – DAY 1

Group	Dose	N	Gender	$C_{0\ hr}$ (µg/mL)	$C_{1\ hr}$ (µg/mL)
2	500 mg/kg/day	5	M	0	1.20
		5	F	0	0.523
		10	Combined Ave.	0	0.861
3	2000 mg/kg/day	5	M	0	2.03
		5	F	0	1.18
		10	Combined Ave.	0	1.61
4	5000 mg/kg/day	5	M	0	2.63
		5	F	0	1.78
		10	Combined Ave.	0	2.21

Table 4.4 Mean Concentrations of XP13512 in Blood of Rats After Oral Dosing of XP13512 – DAY 14

Group	Dose	N	Gender	$C_{0\ hr}$ (µg/mL)	$C_{1\ hr}$ (µg/mL)
2	500 mg/kg/day	5	M	0.428	0.598
		5	F	0.149	0.544
		10	Combined Ave.	0.288	0.571
3	2000 mg/kg/day	5	M	0.244	1.47
		5	F	0.114*	1.48
		10	Combined Ave.	0.186*	1.47
4	5000 mg/kg/day	5	M	0.115	1.42
		5	F	0.0**	1.69
		10	Combined Ave.	0.0719**	1.56

*Data are mean of 4 females and combined average of 9 animals.
**Data are mean of 3 females and combined average of 8 animals.

Study title: **2-Week Oral Gavage Toxicity and Toxicokinetic Study with XP13512 in Rats**

Key study findings: Wistar rats were treated by oral gavage with 0, 500, 2000 or 5000 mg/kg/d for 14 days. Although hypo- and/or hyperactivity was reported with increased incidence in treated animals, there were no adverse effects on other parameters to include body weight, feed consumption, hematology, clinical chemistries and gross necropsy. Increased hyaline droplet formation was observed in treated males at all doses. Therefore, the NOAEL for this study is determined to be <500 mg/kg/d in males and 5000 mg/kg/d in females.

Study no.: 7401-110 or RD2007/01699/00XP007
Volume and page #: Electronic submission
Conducting laboratory and location: (b) (4)
Date of study initiation: 6/5/03
GLP compliance: Yes
QA report: yes
Drug, lot #, and % purity: Lot 2892.A.03.1 at 98.4% purity and 2892.A.03.2 at 97.3% purity

Methods

Doses: 0 (vehicle), 500, 2000 or 5000 mg/kg/d
Species/strain: Wistar (Crl: (WI)BR) rats
Number/sex/group or time point (main study): 10
Route, formulation, volume, and infusion rate: Oral gavage in 0.1% Tween 80® + 0.5% methylcellulose
Satellite groups used for toxicokinetics or recovery: 8/sex/dose for TK
Age: 7-8 weeks of age
Weight: 182- 376 gms
Sampling times: TK samples were taken on Days 1 and 14 at 0, 0.5, 1, 2, 4, 8, 12 and 24 hrs post-dosing.
Unique study design or methodology: Neurobehavioral testing was conducted at 1 hr post-dosing on Day 14.

Observations and times:
Mortality: Twice daily
Clinical signs: Twice daily
Body weights: Weekly
Food consumption: Weekly
Ophthalmoscopy: Prior to dosing and Day 14
Hematology: At termination
Clinical chemistry: At termination
Urinalysis: At termination
Gross pathology: All animals on study
Organ weights: Adrenals, brain, epididymides, heart, kidneys, lung, liver, ovaries, pituitary, prostate, salivary gland, seminal vesicle, spleen, testes, thymus, thyroid/parathyroids, uterus
Histopathology: Adequate Battery: yes for control and high dose only with liver, kidney, pancreas and gross lesions evaluated from other dose groups
Peer review: Not specified

Results
Mortality: There were no premature decedents.

Clinical signs: Adverse clinical signs seen at 1 hr post-dosing included hypoactivity, hyperactivity, recumbency and staining of the hair coat. Hyperactivity was seen in the low and mid dose animals, while the incidence and severity of hypoactivity increased

with increasing dose. Although there was pupillary dilatation in the treated animals when compared to controls, their response to bright light was considered normal. The signs dissipated by the following mornings' evaluations.

Sponsor table:

	NUMBER OF ANIMALS AFFECTED							
SEX:	-------MALE-------				------FEMALE------			
GROUP:	1	2	3	4	1	2	3	4
CATEGORY DOSE:	0	500	2000	5000	0	500	2000	5000
KEYWORD / QUALIFIER NUMBER:	10	10	10	10	10	10	10	10
*** TOP OF LIST ***								
APPEARANCE								
BENT TAIL	0	0	0	0	0	0	1	0
MALOCCLUSION	0	0	1	0	1	0	0	0
BEHAVIOR								
HYPOACTIVE	1	7	9	10	0	5	5	8
HYPERACTIVE	0	5	7	1	0	10	10	8
RECUMBENT								
LATERAL	0	1	0	1	0	0	0	0
STERNAL	0	8	9	6	0	6	1	3
SENSITIVE TO TOUCH	0	1	0	0	0	0	0	1
DISCHARGE								
CLEAR-ORAL	0	0	0	1	0	0	0	1
RED-ORAL	0	0	0	1	0	0	0	0
EYES								
PROTRUDING								
EYE-LEFT	0	0	0	0	1	0	0	0
RESPIRATION								
AUDIBLE	0	0	0	4	0	1	0	3
SKIN & PELAGE								
BROWN HAIR COAT								
PERINEAL AREA	0	0	0	1	0	0	0	0

Body weights: No intergroup differences were discerned.

Food consumption: No treatment-related differences were determined.

Ophthalmoscopy: No treatment-engendered lesions were discovered.

Hematology: No consistent treatment-related adverse effects were described.

Clinical chemistry: There were no significant intergroup, treatment-related effects.

Urinalysis: Although increased urine volume and slightly higher urine pH were found in mid and high dose males and all treated females, the biological significance of these findings is uncertain as renal histologic lesions were found only in treated males.

Gross pathology: No consistent treatment-related lesions were described.

Organ weights: Kidney weights were significantly increased (≤10%) in the mid and high dose males. Histologic correlates were found.

Histopathology: Adequate Battery: yes
Peer review: Not specified
Increased hyaline droplet formation was noted in the renal tubular epithelium of all treated males.
Sponsor tables:

		--- N U M B E R - O F - A N I M A L S - A F F E C T							
TABLE INCLUDES: SEX=ALL;GROUP=1,2,3,4;WEEKS=ALL; DEATH=T;FIND=ALL;SUBSET=ALL	SEX:	-------MALE-------				------FEMALE------			
	GROUP:	-1-	-2-	-3-	-4-	-1-	-2-	-3-	-4-
ORGAN AND FINDING DESCRIPTION	NUMBER:	10	10	10	10	10	10	10	10
** TOP OF LIST **									
KIDNEY (KD)	NUMBER EXAMINED:	10	10	10	10	10	10	10	10
	NOT REMARKABLE:	10	1	0	0	0	5	3	3
--HYALINE DROPLETS, TUBULAR EPITHELIUM		0	9	10	10	0	0	0	0
--REGENERATION, TUBULAR EPITHELIUM		0	3	4	6	4	3	3	5
--DILATATION, PELVIC		0	0	0	1	0	0	0	0
--INFLAMMATION, CHRONIC		0	0	0	0	3	0	1	0
--MICROCONCRETIONS, TUBULE		0	0	0	0	5	3	6	6
--PYELITIS		0	0	0	0	2	1	1	1
--HYPERPLASIA, UROTHELIUM		0	0	0	0	1	0	1	1

		--- N U M B E R - O F - A N I M A L S - A F F E C							
TABLE INCLUDES: SEX=ALL;GROUP=1,2,3,4;WEEKS=ALL; DEATH=T;FIND=ALL;SUBSET=ALL	SEX:	-------MALE-------				------FEMALE------			
	GROUP:	-1-	-2-	-3-	-4-	-1-	-2-	-3-	-4-
ORGAN AND FINDING DESCRIPTION	NUMBER:	10	10	10	10	10	10	10	10
** TOP OF LIST **									
KIDNEY (KD)	NUMBER EXAMINED:	10	10	10	10	10	10	10	10
	NOT REMARKABLE:	10	1	0	0	0	5	3	3
--HYALINE DROPLETS, TUBULAR EPITHELIUM		0	9	10	10	0	0	0	0
--REGENERATION, TUBULAR EPITHELIUM		0	3	4	6	4	3	3	5
--DILATATION, PELVIC		0	0	0	1	0	0	0	0
--INFLAMMATION, CHRONIC		0	0	0	0	3	0	1	0
--MICROCONCRETIONS, TUBULE		0	0	0	0	5	3	6	6
--PYELITIS		0	0	0	0	2	1	1	1
--HYPERPLASIA, UROTHELIUM		0	0	0	0	1	0	1	1

Toxicokinetics: Effective hydrolysis of XP13512 to gabapentin was evident at all doses. Gabapentin concentrations in blood of 500 mg/kg/d animals and the 2000 mg/kg/d females declined after Cmax was reached (in ≤8 hrs).

The 5000 mg/kg/d dose concentration of gabapentin in blood at 24 hrs was higher than that found at 12 hrs post-dosing so the sponsor excluded these data from their calculations. This was done as the sponsor believed that the finding "may indicate an error in either the sample collection or analysis order".

Absorption may have been delayed at the 5000 mg/kg/d dose as there was a more linear relationship to dose on Day 14 than on Day 1. Tmax and $t_{1/2}$ increased slightly over the 14 day dosing period, but no evidence of drug accumulation (prodrug, gabapentin or gabapentin-lactam) was demonstrated.

Sponsor tables:

Table 4.1 Toxicokinetics of Gabapentin in Blood Following the First and Last Doses in a 14-Day Repeated Dose Toxicity Study of Oral XP13512 in Rats

Dose (mg/kg)	Day	Gender	Pharmacokinetic Parameters for Gabapentin				
			Cmax (μg/mL)	Tmax (hr)	T1/2 (hr)	AUC(0-last) (μg.hr/mL)	AUC(0-inf) (μg.hr/mL)
500	1	Male	79.0	1.00	2.16	497	497
		Female	114	0.50	2.11	423	423
		Combined	96.4	0.75	2.13	460	460
500	14	Male	139	0.50	2.16	546	547
		Female	120	0.50	2.35	418	418
		Combined	130	0.50	2.25	482	482
2000	1	Male	164	8.00	11.3	2080	2830
		Female	134	2.00	5.13	1410	1480
		Combined	149	5.00	8.23	1740	2160
2000	14	Male	204	4.00	2.44	2060	2060
		Female	175	0.50	2.15	1610	1610
		Combined	189	2.25	2.29	1840	1840
5000	1	Male	207	4.00	5.11[1]	1790[1]	2310[1]
		Female	172	0.50	5.27[1]	1370[1]	1770[1]
		Combined	189	2.25	5.19[1]	1580[1]	2040[1]
5000	14	Male	375	4.00	3.48	4840	4910
		Female	340	4.00	3.11	3630	3660
		Combined	357	4.00	3.29	4230	4280

[1]Data for 24 hours were excluded from calculation of pharmacokinetic parameters (see section 2.6).

Table 4.2 Toxicokinetics of Gabapentin-Lactam in Blood Following the First and Last Doses in a 14-Day Repeated Dose Toxicity Study of Oral XP13512 in Rats

Dose (mg/kg)	Day	Gender	Pharmacokinetic Parameters for Gabapentin-Lactam				
			Cmax (µg/mL)	Tmax (hr)	T1/2 (hr)	AUC(0-last) (µg.hr/mL)	AUC(0-inf) (µg.hr/mL)
500	1	Male	1.70	1.00	5.18	16.5	17.4
		Female	2.17	2.00	2.67	18.0	18.1
		Combined	1.93	1.50	3.92	17.3	17.7
500	14	Male	2.26	4.00	4.85	17.7	18.2
		Female	2.71	1.00	3.62	19.1	19.3
		Combined	2.48	2.50	4.24	18.4	18.7
2000	1	Male	4.20	8.00	10.4	59.5	78.3
		Female	3.93	8.00	5.57	65.9	70.6
		Combined	4.07	8.00	7.97	62.7	74.5
2000	14	Male	4.93	4.00	3.61	58.3	59.3
		Female	5.79	4.00	5.84	72.6	78.3
		Combined	5.36	4.00	4.72	65.4	68.8
5000	1	Male	6.29	4.00	9.68[1]	55.5[1]	105[1]
		Female	6.11	8.00	ND[1]	52.9[1]	ND[1]
		Combined	6.20	6.00	9.68[1]	54.2[1]	105[1]
5000	14	Male	8.43	4.00	3.92	113	115
		Female	9.54	8.00	4.40	118	123
		Combined	8.99	6.00	4.16	115	119

ND – Not determined due to lack of a defined elimination phase.
[1]Data for 24 hours were excluded from calculation of pharmacokinetic parameters (see section 2.6).

Table 4.3 Toxicokinetics of XP13512 in Blood Following the First and Last Doses in a 14-Day Repeated Dose Toxicity Study of Oral XP13512 in Rats

Dose (mg/kg)	Day	Gender	Pharmacokinetic Parameters for XP13512				
			Cmax (µg/mL)	Tmax (hr)	T1/2 (hr)	AUC(0-last) (µg.hr/mL)	AUC(0-inf) (µg.hr/mL)
500	1	Male	0.468	1.00	1.72	1.55	1.61
		Female	0.734	0.50	1.39	1.13	1.37
		Combined	0.601	0.75	1.56	1.34	1.49
500	14	Male	1.12	0.50	1.09	1.59	1.59
		Female	1.47	0.50	1.25	2.03	2.06
		Combined	1.30	0.50	1.17	1.81	1.82
2000	1	Male	0.915	0.50	12.3	10.2	14.1
		Female	0.977	0.50	5.65	6.96	7.49
		Combined	0.946	0.50	8.95	8.58	10.8
2000	14	Male	1.36	0.50	1.62	7.82	7.90
		Female	3.12	0.50	4.39	8.87	9.28
		Combined	2.24	0.50	3.01	8.35	8.59
5000	1	Male	1.61	0.50	7.14[1]	9.91[1]	14.1[1]
		Female	1.75	0.50	6.28[1]	6.52[1]	9.62[1]
		Combined	1.68	0.50	6.71[1]	8.22[1]	11.9[1]
5000	14	Male	3.05	4.00	3.61	24.3	24.6
		Female	2.30	4.00	4.51	18.3	19.0
		Combined	2.67	4.00	4.06	21.3	21.8

[1]Data for 24 hours were excluded from calculation of pharmacokinetic parameters (see section 2.6).

Other: Neurobehavioral testing revealed hyperactivity in treated males and hypoactivity in treated females when compared to controls. Treated animals were also considered more reactive to handling than the controls. When the animals were evaluated in the open field test, locomotion was higher in low and mid dose animals when compared to controls and high dose animals. Dilated pupils were more frequently reported in treated animals and treated males had a dose-related increase in muscle tone.

Study title: **13-Week Oral Toxicity Study of XP13512 in Rats**

Key study findings: Due to the hypoactivity in all XP13512-treated animals with increased salivation coupled with the centrilobular hypertrophy in the liver at 5000 mg/kg/d and the hyaline droplet and chronic progressive nephropathy in the 2000 and 5000 mg/kg/d males, no NOAEL was determined in this 13-week study in Wistar rats.

Study no.: 1032-009 or RD2007/01523/00
Volume and page #: Electronic submission
Conducting laboratory and location: (b) (4)
Date of study initiation: 6/24/04

GLP compliance: Yes
QA report: yes
Drug, lot #, and % purity: Lots 3, 5 at 99.5% purity

Methods

Doses: 0 (vehicle), 500, 2000 or 5000 mg/kg/d
Species/strain: Wistar [Crl: WI)BR] rats
Number/sex/group or time point (main study): 15
Route, formulation, volume, and infusion rate: Oral gavage in 0.1% Tween 80® and 0.5% methylcellulose at 20 mL/kg
Satellite groups used for toxicokinetics or recovery: 15/sex/dose
Age: 8 weeks of age at study initiation
Weight: 163- 280 gms at study initiation
Sampling times: TK samples were taken from the orbital sinus on Days 1 and 90 at pre-dosing, 0.5, 1, 2, 4, 8, 12 and 24 hrs post-dosing (N=3/sex/time point)

Observations and times:
Mortality and clinical signs: Twice daily
Body weights: Weekly
Food consumption: Weekly
Ophthalmoscopy: Prior to dosing and prior to necropsy
EKG: Not performed
Hematology: At terminal necropsy
Clinical chemistry: As for hematology
Urinalysis: As for hematology
Gross pathology: All animals from the main study
Organ weights: Adrenals, brain, ovaries, testes, kidneys, heart, liver, lung, pituitary, prostate, seminal vesicles, mandibular salivary gland, thymus, spleen, thyroid/parathyroids, uterus
Histopathology: Adequate Battery: yes for control and high dose animals; liver, kidney and gross lesions only from other groups
Peer review: Not specified

Results
Mortality: There were no premature decedents other than one high dose male (#1039) that aspirated the test preparation.

Clinical signs: Hypoactivity was noted in mid and high dose animals with increased salivation. No other consistent adverse signs were described.

Body weights: Body weights of treated groups were higher than those of controls.

Food consumption: Consumption was higher in treated groups than in controls.

Ophthalmoscopy: No treatment-related ocular lesions were engendered.

Hematology: No significant intergroup differences were determined.

Clinical chemistry: In the high dose females, cholesterol levels were increased but the biological significance of this finding is uncertain as it is frequently observed in rats.

Urinalysis: No meaningful intergroup differences were found.

Gross pathology: No consistent gross lesions were seen as attributable to treatment.

Organ weights: Liver weights were moderately increased in mid and high dose animals (16-36% in males; 10-27% in females). Histologic correlates were found.

Kidney weights were increased in mid dose males and both sexes at the 5000 mg/kg/d dose. Histologic correlates were found in males at all doses, but no correlates were found in the females.

Although thyroid weights were increased in mid dose females and both sexes at 5000 mg/kg/d, no histologic correlates were described.

Sponsor table:

Summary of Organ Weight Values - MALE

Table 8 Cont. Terminal

	0 mg/kg/day (Control)			500 mg/kg/day			2000 mg/kg/day			5000 mg/kg/day		
Endpoint	Mean	SD	N	Mean	SD	N	Mean	SD	N	Mean	SD	N
Kidneys												
g	3.426	0.236	15	3.485	0.485	15	3.896[a]	0.443	14	4.684[b]	0.661	15
Kidneys/BWt %	0.7334	0.0380	15	0.7181	0.1056	15	0.7848	0.0642	14	0.9543[b]	0.0976	15
Kidneys/BrWt ratio	1.7555	0.1493	15	1.7870	0.2675	15	2.0158[a]	0.2220	14	2.5040[b]	0.4114	15
Liver												
g	13.217	1.185	15	14.312	1.644	15	15.379[b]	1.400	14	18.006[b]	2.161	15
Liver/BWt %	2.8249	0.1394	15	2.9296	0.2022	15	3.0979[b]	0.1346	14	3.6699[b]	0.3019	15
Liver/BrWt ratio	6.7670	0.6414	15	7.3347	0.8935	15	7.9491[b]	0.5680	14	9.5963[b]	1.1249	15
Lung												
g	1.822	0.217	15	1.868	0.196	15	1.828	0.173	14	1.943	0.245	15
Lung/BWt %	0.3903	0.0449	15	0.3830	0.0306	15	0.3690	0.0301	14	0.3961	0.0368	15
Lung/BrWt ratio	0.9351	0.1334	15	0.9584	0.1191	15	0.9468	0.0927	14	1.0367	0.1351	15

N - Number of measures used to calculate mean
SD - Standard Deviation
[a]Significantly different from control; ($p<0.05$)
[b]Significantly different from control; ($p<0.01$)

Table 8 Cont. **Summary of Organ Weight Values - FEMALE**
Terminal

Endpoint	0 mg/kg/day (Control) Mean	SD	N	500 mg/kg/day Mean	SD	N	2000 mg/kg/day Mean	SD	N	5000 mg/kg/day Mean	SD	N
Kidneys												
g	2.009	0.136	15	2.064	0.174	15	2.072	0.165	15	2.264[b]	0.212	15
Kidneys/BWt %	0.7619	0.0629	15	0.7510	0.0336	15	0.7830	0.0493	15	0.8193[a]	0.0587	15
Kidneys/BrWt ratio	1.1012	0.0856	15	1.1410	0.0895	15	1.1874	0.1288	15	1.2888[b]	0.1234	15
Liver												
g	7.573	0.778	15	7.980	0.814	15	8.326[a]	0.701	15	9.612[b]	0.797	15
Liver/BWt %	2.8628	0.2192	15	2.9014	0.1725	15	3.1449[b]	0.1998	15	3.4807[b]	0.2294	15
Liver/BrWt ratio	4.1466	0.4172	15	4.4142	0.4549	15	4.7558[b]	0.3521	15	5.4712[b]	0.4584	15
Lung												
g	1.376	0.084	14	1.400	0.153	14	1.394	0.123	15	1.422	0.166	15
Lung/BWt %	0.5198	0.0354	14	0.5091	0.0402	14	0.5268	0.0414	15	0.5145	0.0520	15
Lung/BrWt ratio	0.7530	0.0404	14	0.7740	0.0731	14	0.7975	0.0805	15	0.8103	0.1037	15

N - Number of measures used to calculate mean
SD - Standard Deviation
[a]Significantly different from control; ($p<0.05$)
[b]Significantly different from control; ($p<0.01$)

Histopathology: Adequate Battery: yes
Peer review: Not specified

Minimal to mild centrilobular hepatocyte hypertrophy was appreciated in high dose animals (8 males, 13 females) and is considered attributable to the test article. The other male dose groups showed minimal centrilobular hepatocellular vacuolation.

Hyaline droplets were found in renal tubular epithelia in increased incidence in treated males (9 low dose males and all males at higher doses) and minimal to mild chronic progressive nephropathy was noted in mid and high dose males.

The premature decedent showed evidence of test article aspiration. Two additional males and one female from the 5000 mg/kg/d groups also had lesions consistent with aspiration (hyperplasia of the respiratory epithelium with ulceration, inflammation and hemorrhage) but had no clinical signs.

Toxicokinetics: Exposure to gabapentin increased with increasing dose. AUC values on Day 90 were comparable to those on Day 1, but exposures were slightly higher.

Sponsor tables:

Table 4.1 Toxicokinetic Parameters for Gabapentin in Blood of Rats After Oral Dosing of XP13512 – DAY 1

Group	Dose	Gender	C_{max} (µg/mL)	T_{max} (hr)	$T_{1/2}$ (hr)	AUC_{last} (hr*µg/mL)	$AUC_{(0\text{-}inf)}$ (hr*µg/mL)
5	500 mg/kg/day	F	80.8	0.50	2.50	329	330
		M	74.8	1.00	3.10	391	393
		Combined Ave.	77.8	0.75	2.80	360	362
6	2000 mg/kg/day	F	141	8.00	6.93	1560	1780
		M	154	4.00	4.91	1520	1590
		Combined Ave.	147	6.00	5.92	1540	1690
7	5000 mg/kg/day	F	167	4.00	11.9	2070	2910
		M	271	1.00	5.69	2580	2710
		Combined Ave.	219	2.50	8.81	2320	2810

Table 4.1 Toxicokinetic Parameters for Gabapentin in Blood of Rats After Oral Dosing of XP13512 – DAY 1

Group	Dose	Gender	C_{max} (µg/mL)	T_{max} (hr)	$T_{1/2}$ (hr)	AUC_{last} (hr*µg/mL)	$AUC_{(0\text{-}inf)}$ (hr*µg/mL)
5	500 mg/kg/day	F	80.8	0.50	2.50	329	330
		M	74.8	1.00	3.10	391	393
		Combined Ave.	77.8	0.75	2.80	360	362
6	2000 mg/kg/day	F	141	8.00	6.93	1560	1780
		M	154	4.00	4.91	1520	1590
		Combined Ave.	147	6.00	5.92	1540	1690
7	5000 mg/kg/day	F	167	4.00	11.9	2070	2910
		M	271	1.00	5.69	2580	2710
		Combined Ave.	219	2.50	8.81	2320	2810

Table 4.2 Toxicokinetic Parameters for Gabapentin in Blood of Rats After Oral Dosing of XP13512 – DAY 90

Group	Dose	Gender	C_{max} (µg/mL)	T_{max} (hr)	$T_{1/2}$ (hr)	AUC_{last} (hr*µg/mL)	$AUC_{(0\text{-}24)}$ (hr*µg/mL)
5	500 mg/kg/day	F	152	0.50	3.20	450	450
		M	130	0.50	3.31	474	474
		Combined Ave.	141	0.50	3.25	462	462
6	2000 mg/kg/day	F	251	1.00	5.00	1540	1540
		M	253	2.00	5.27	1640	1640
		Combined Ave.	252	1.50	5.13	1590	1590
7	5000 mg/kg/day	F	415	2.00	5.78	4960	4960
		M	357	2.00	3.49	5620	5620
		Combined Ave.	386	2.00	4.64	5290	5290

Table 4.3 Toxicokinetic Parameters for XP13512 in Blood of Rats After Oral Dosing of XP13512 – DAY 1

Group	Dose	Gender	C_{max} (µg/mL)	T_{max} (hr)	$T_{1/2}$ (hr)	AUC_{last} (hr*µg/mL)	$AUC_{(0\text{-inf})}$ (hr*µg/mL)
5	500 mg/kg/day	F	0.537	0.50	1.85	0.767	0.841
		M	0.380	0.50	9.83	1.13	2.95
		Combined Ave.	0.459	0.50	5.84	0.951	1.89
6	2000 mg/kg/day	F	0.665	1.00	7.15	6.00	6.77
		M	0.885	2.00	5.35	6.61	6.98
		Combined Ave.	0.775	1.50	6.25	6.30	6.87
7	5000 mg/kg/day	F	1.56	0.50	7.74	10.1	12.1
		M	1.36	2.00	4.20	10.3	10.5
		Combined Ave.	1.46	1.25	5.97	10.2	11.3

Table 4.4 Toxicokinetic Parameters for XP13512 in Blood of Rats After Oral Dosing of XP13512 – DAY 90

Group	Dose	Gender	C_{max} (µg/mL)	T_{max} (hr)	$T_{1/2}$ (hr)	AUC_{last} (hr*µg/mL)	$AUC_{(0\text{-}24)}$ (hr*µg/mL)
5	500 mg/kg/day	F	0.988	4.00	ND	3.92	3.97
		M	0.591	0.50	2.46	0.991	1.24
		Combined Ave.	0.789	2.25	2.46	2.46	2.60
6	2000 mg/kg/day	F	3.17	0.50	3.81	8.69	8.69
		M	1.34	1.00	4.15	5.31	5.31
		Combined Ave.	2.25	0.75	3.98	7.00	7.00
7	5000 mg/kg/day	F	3.16	2.00	5.19	32.8	32.8
		M	1.76	2.00	2.18	26.3	26.3
		Combined Ave.	2.46	2.00	3.68	29.5	29.5

ND – Not determined due to insufficient quantifiable data points.

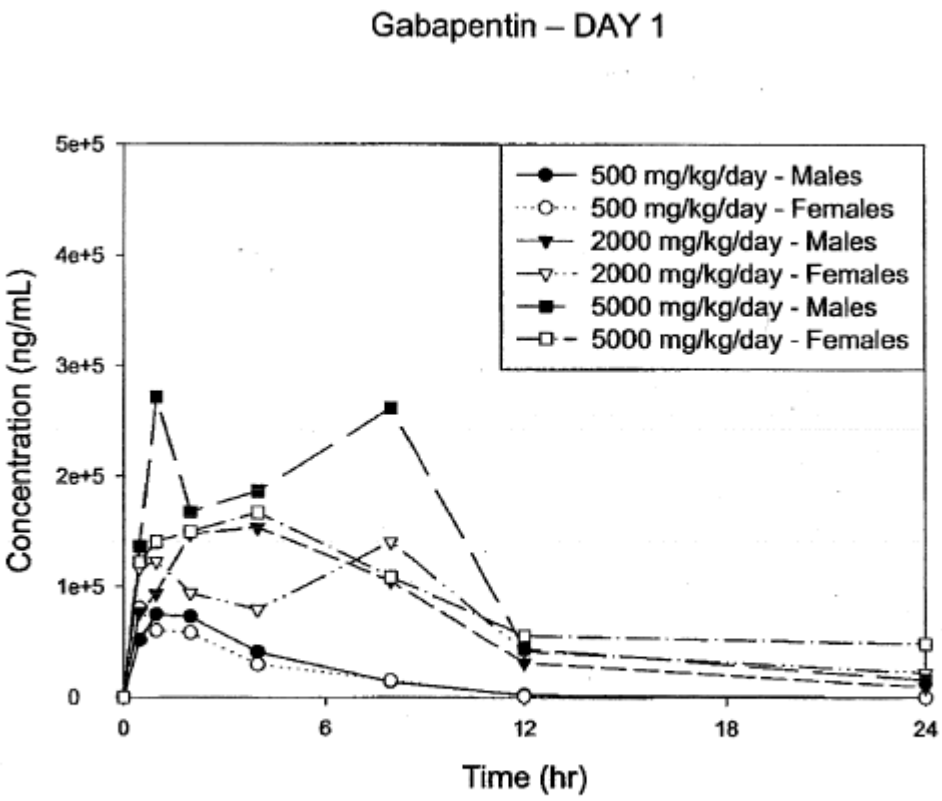


Figure 5.1 Concentrations of gabapentin in blood of male and female rats following the first oral dose of XP13512 at 500, 2000, and 5000 mg/kg/day (Day 1).

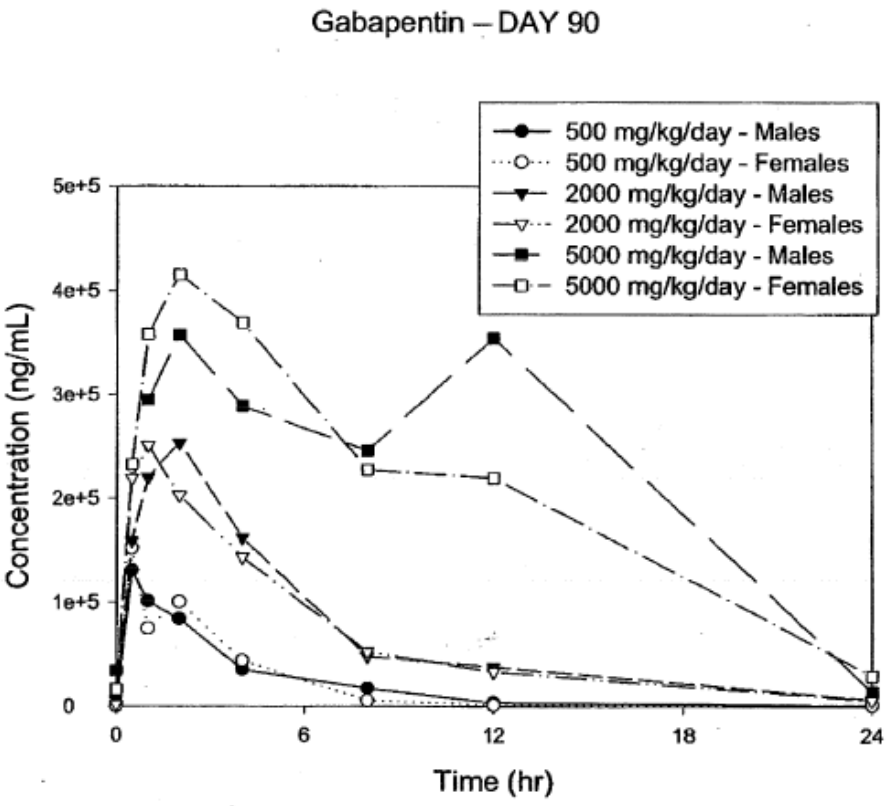


Figure 5.2 Concentrations of gabapentin in blood of male and female rats following the last oral dose of XP13512 at 500, 2000, and 5000 mg/kg/day (Day 90).

Study title: **6-Month Oral Toxicity Study of XP13512 in Rats with a 1-Month Recovery Period**

Key study findings: In this 6-month oral gavage study in Wistar rats, the doses were 0, 500, 2000 or 5000 mg/kg/d. As in the previous shorter term rat studies, increased hyaline droplet formation was noted in the treated male groups. Reversal was incomplete at the end of the recovery period. Centrilobular hepatocellular hypertrophy was described in the high dose animals, but was reversed by the end of the 1 month recovery period. No NOAEL for the histologic renal findings was found in this study, but clinical chemistries and urinalyses were not affected.

Study no.: 1032-034 or RD2007/01526/00XP046
Volume and page #: Electronic submission
Conducting laboratory and location: (b) (4)
Date of study initiation: 1/25/05
GLP compliance: Yes
QA report: yes
Drug, lot #, and % purity: Batch 10 at >99% purity

Methods

Doses: 0 (vehicle), 500, 2000 or 5000 mg/kg/d
Species/strain: Wistar (Crl:WI) rats
Number/sex/group or time point (main study): 25
Route, formulation, volume, and infusion rate: Oral gavage in 0.1% Tween 80® + 0.5% methylcellulose at 20 mL/kg
Satellite groups used for toxicokinetics or recovery: 12/sex/group from treated groups for TK analysis on Day 1 only; 3-5 survivors from main study for recovery
Age: 8 weeks of age at study initiation
Weight: 161- 274 gms
Sampling times: TK samples were taken from satellite animals (N=3/time point) on Day 1 (pre-dose), 1, 2, 4, 6, 10 and 24 hrs post-dosing and main study animals on Day 175 (N= 18/sex/main study group) at 1, 2, 4, 6, 10 and 24 hrs post-dosing.

Observations and times:

Mortality: Twice daily
Clinical signs: Weekly
Body weights: Weekly
Food consumption: Weekly
Ophthalmoscopy: Prior to dosing and prior to necropsy for main study animals
EKG: Not performed
Hematology: At terminal necropsy and end of recovery period
Clinical chemistry: As for hematology
Urinalysis: As for hematology from cage pan samples
Gross pathology: All study animals
Organ weights: Adrenals, brain, ovaries, testes, kidneys, heart, liver, lung, pituitary, prostate, seminal vesicles, mandibular salivary gland, thymus, spleen, thyroid/parathyroids, uterus
Histopathology: Adequate Battery: yes, from control and high dose only; mandibular salivary gland (all main study animals), liver, and kidney were examined at recovery necropsy.

Peer review: Not specified

Results

Mortality: Accidental deaths occurred at every dose level (2 male controls, 1 male/2 females from the low dose group, 4 males from the mid dose group, 1 male and 5 females from the high dose group).

Clinical signs: Hypoactivity was not reported for this study as had been found consistently in earlier studies. The lack is significant, but no explanation was provided.

Increased salivation with rales and/or audible breathing was observed in several high dose animals.

Body weights: Weights were increased in the 5000 mg/kg/d animals when compared to controls.

Food consumption: Consumption was increased in the 5000 mg/kg/d animals.

Ophthalmoscopy: No treatment-engendered lesions were described.

Hematology: As in the shorter term studies, rbc parameters (hematocrit, hemoglobin) were slightly decreased in the high dose males. These animals also had slightly increased neutrophil counts.

Clinical chemistry: No consistent intergroup differences were discussed.

Urinalysis: No treatment-related effects were determined.

Gross pathology: No treatment-attributable gross lesions were described.

Organ weights: Kidney weights were increased in the mid (19%) and high dose males (44%) when compared to controls and histologic correlates were appreciated. Increased liver weights were determined in the 5000 mg/kg/d animals of both sexes (36% for males; 24% for females). Histologic correlates were found. By the end of the recovery period, absolute kidney weights were 14% higher in 5000 mg/kg/d males compared to controls. However, increased absolute liver weights were also noted in the 500 mg/kg/d males and both sexes at 2000 mg/kg/d (17% for males; 8% for females), but no histologic correlates were found in these groups. By the end of the recovery period, the liver weights were comparable across groups.

The mandibular salivary glands were decreased in weight in mid and high dose animals, but the only histologic correlate was slight depletion of the secretory granular ducts.

Histopathology: Adequate Battery: yes
Peer review: Not specified

As in the shorter term rat studies, increased hyaline droplet formation was seen in all treated males with increased incidence and severity in the mid and high dose groups. At the end of the recovery period, increased droplets were found in a few 5000 mg/kg/d males. The location of the droplets and the differences between the males and females would indicate that ά2-μ globulin played a role in the droplet formation. No tubular necrosis was found accompanying the droplets.
For mains study males:

Sponsor tables:

kidneys		(2)	(20)	(1)	(20)	(4)	(18)	(1)	(2
hyaline, droplets, increased		0	0	0	19	2	18	1	2
	- minimal	0	0	0	4	1	0	0	
	- mild	0	0	0	15	1	0	0	
	- moderate	0	0	0	0	0	18	1	2
hydronephrosis, unilateral		0	0	0	0	0	1	0	
	- minimal	0	0	0	0	0	0	0	
	- mild	0	0	0	0	0	1	0	
hyperplasia, transitional cell	- mild	0	0	0	0	1	0	0	
mineralization, pelvic		0	0	0	3	1	1	0	
	- minimal	0	0	0	3	0	1	0	
	- mild	0	0	0	0	1	0	0	
mineralization, tubular	- minimal	0	5	0	2	0	0	0	
nephropathy, chronic progressive		0	11	1	16	1	15	1	1
	- minimal	0	11	1	16	1	14	1	1
	- mild	0	0	0	0	0	1	0	
pyelitis	- minimal	0	0	0	0	0	1	0	
within normal limits		2	6	0	0	1	0	0	
lacrimal glands, exorbital		(2)	(20)	(1)	(0)	(4)	(0)	(1)	(2
infiltration, lymphocytic	- minimal	0	1	0	0	0	0	0	
metaplasia, harderian		2	13	1	0	2	0	1	
	- minimal	2	11	1	0	1	0	1	
	- mild	0	2	0	0	1	0	0	

DOS - Died or euthanized on study
SNC - Scheduled necropsy
( ) - Number observed

For recovery males:

kidneys		(3)	(4)	(3)	(4)
hyaline, droplets, increased	- minimal	0	0	0	2
mineralization, pelvic	- minimal	0	0	1	0
mineralization, tubular	- minimal	3	0	0	3
nephropathy, chronic progressive	- minimal	2	3	3	3
within normal limits		0	1	0	0

For main study females:

kidneys		(0)	(20)	(2)	(20)	(0)	(20)	(5)	(17)
cyst	- mild	0	1	0	0	0	0	0	0
hyperplasia, transitional cell	- minimal	0	2	0	0	0	2	0	1
mineralization, pelvic	- minimal	0	6	0	3	0	8	0	4
mineralization, tubular	- minimal	0	10	1	8	0	11	0	5
nephropathy, chronic progressive	- minimal	0	3	0	5	0	5	0	6

DOS - Died or euthanized on study
SNC - Scheduled necropsy
( ) - Number observed

Minimal centrilobular hepatocyte hypertrophy was seen in both sexes (17/20 males; 14/17 females) at terminal necropsy at 5000 mg/kg/d. No hepatic alterations were appreciated at the recovery sacrifice.

Toxicokinetics: Hydrolysis of XP13512 to gabapentin was obvious at all dose levels and exposures increased with increasing dose levels and similar on Days 1 and 175.

Sponsor table:

Whole Blood Exposure of XP13512 and Gabapentin After Oral Administration of XP13512 in Rats

Pharmacokinetic Parameter	XP13512 Dose (mg/kg)	Gabapentin		XP13512	
		Day 1	Day 175	Day 1	Day 175
AUC (µg·hr/mL)	500	322	362	1.16	2.22
	2000	805	1470	4.18	9.00
	5000	4170	3130	41.2	22.6
C_{max} (µg/mL)	500	64.4	105	0.335	0.705
	2000	103	206	0.982	1.81
	5000	208	345	2.09	2.70
T_{max} (hr)	500	1.00	1.00	1.00	2.50
	2000	3.50	1.50	3.50	3.50
	5000	3.00	2.50	3.00	3.00

AUC = Area under the concentration vs. time curve ($AUC_{(0\text{-}inf)}$ on Day 1 and $AUC_{(0\text{-}24)}$ on Day 175)
C_{max} = Concentration maximum
T_{max} = Time to C_{max}.

Study title: **13-Week Oral Toxicity Study of XP13512 in Monkeys**

Key study findings: There were no adverse findings in any of the parameters evaluated in cynomolgus monkeys treated for 13 weeks at doses of 500, 1000 or 2000 mg/kg/d. The Cmax and AUC values at the 2000 mg/kg/d dose were 278 µg/mL and 2670 µg.h/mL for gabapentin and 15.6 µg/mL and 76.4 µg.h/mL for XP13512. This appears to have been neither an MTD nor an MFD dose study but compliant with the OECD and ICH M3 R2 recommendations

Study no.: 1032-008 or RD2007/01524/00XP026
Volume and page #: Electronic submission
Conducting laboratory and location: (b) (4)
Date of study initiation: 6/25/04
GLP compliance: Yes
QA report: yes
Drug, lot #, and % purity: Batch 5 at 99.6% purity

Methods

Doses: 0 (vehicle), 500, 1000, or 2000 mg/kg/d
Species/strain: Cynomolgus monkeys originally from (b) (4)
Number/sex/group or time point (main study): 4

Route, formulation, volume, and infusion rate: Oral gavage in 0.1% Tween 80® + 0.5% methylcellulose at 10 mL/kg

Satellite groups used for toxicokinetics or recovery: None

Age: 22- 31 months of age

Weight: 1.78- 2.05 kg for males; 1.74- 2.08 kg for females

Sampling times: TK samples were taken on Days 1 and 90 at 1, 2, 4, 6, 8, 12 and 24 hrs post-dosing

Observations and times:

Mortality: Twice daily
Clinical signs: Twice daily
Body weights: Weekly
Food consumption: "Availability checked twice daily"
Ophthalmoscopy: Prior to dosing and at study termination
EKG: Prior to dosing and at study termination
Hematology: Prior to dosing and at study termination
Clinical chemistry: Prior to dosing and at study termination
Urinalysis: Prior to dosing and at study termination
Gross pathology: All animals on study
Organ weights: Adrenals, brain, ovary, testes, heart, kidneys, liver, lung, salivary gland, prostate, spleen, thymus, thyroid/parathyroids, uterus
Histopathology: Adequate Battery: yes
Peer review: Not specified

Results

Mortality: There were no premature decedents attributed to the test article. Animal #128 (male, 2000 mg/kg/d) was found dead on D 20 and was determined to have died from a gavage error. A female from the 500 mg/kg/d group (#113) was found dead on D 32 but no cause of death was determined.

Clinical signs; Body weights; Food consumption; Ophthalmoscopy; EKG; Hematology; Clinical chemistry; Urinalysis; Gross pathology; Organ weights: There were no treatment-attributable adverse effects on any of the parameters assessed.

Histopathology: Adequate Battery: yes
Peer review: Not specified
No treatment-engendered lesions were described.

Toxicokinetics: Exposure to gabapentin increased with increasing doses of XP13512. Exposures were comparable on Days 1 and 90.

Sponsor table:

Whole Blood Exposure of XP13512 and Gabapentin After Oral Administration of XP13512 in Monkeys					
Pharmacokinetic Parameter	XP13512 Dose (mg/kg/day)	Gabapentin		XP13512	
		Day 1	Day 90	Day 1	Day 90
AUC (µg·hr/mL)	500	791	775	6.98	10.8
	1000	1790	1550	15.6	22.3
	2000	3240	2670	76.4	54.4
C_{max} (µg/mL)	500	117	115	3.89	5.13
	1000	191	231	5.31	11.8
	2000	321	278	18.7	15.6
T_{max} (hr)	500	1.50	1.43	1.00	1.14
	1000	2.50	1.75	1.25	1.00
	2000	2.75	3.14	1.75	1.14
AUC = Area under the concentration vs. time curve ($AUC_{(0\text{-}inf)}$ on Day 1 and $AUC_{(0\text{-}24)}$ on Day 90) C_{max} = Concentration maximum T_{max} = Time to Cmax					

Table 4.1 Toxicokinetic Parameters for Gabapentin in Blood of Monkeys After Oral Dosing of XP13512 – DAY 1

Group	Dose	Gender	N	C_{max} (µg/mL)	T_{max} (hr)	$T_{1/2}$ (hr)	AUC_{last} (µg*hr/mL)	$AUC_{(0\text{-}inf)}$ (µg*hr/mL)
2	500 mg/kg/day	F	4	130	1.25	3.33	826	853
		M	4	104	1.75	3.13	725	729
		Combined Ave.	8	117	1.50	3.23	775	791
3	1000 mg/kg/day	F	4	191	2.50	3.28	1900	1920
		M	4	191	2.50	3.38	1640	1660
		Combined Ave.	8	191	2.50	3.33	1770	1790
4	2000 mg/kg/day	F	4	307	2.50	6.96	3080	3380
		M	4	335	3.00	3.31	3070	3090
		Combined Ave.	8	321	2.75	5.14	3070	3240

Table 4.2 Toxicokinetic Parameters for Gabapentin in Blood of Monkeys After Oral Dosing of XP13512 – DAY 90

Group	Dose	Gender	N	C_{max} (µg/mL)	T_{max} (hr)	$T_{1/2}$ (hr)	AUC_{last} (µg*hr/mL)	$AUC_{(0-24)}$ (µg*hr/mL)
2	500 mg/kg/day	F	3	116	2.00	3.12	785	785
		M	4	115	1.00	3.22	767	767
		Combined Ave.	7	115	1.43	3.18	775	775
3	1000 mg/kg/day	F	4	229	1.75	3.32	1600	1600
		M	4	232	1.75	3.14	1500	1500
		Combined Ave.	8	231	1.75	3.23	1550	1550
4	2000 mg/kg/day	F	4	279	4.00	3.35	2810	2810
		M	3	275	2.00	3.34	2490	2490
		Combined Ave.	7	278	3.14	3.35	2670	2670

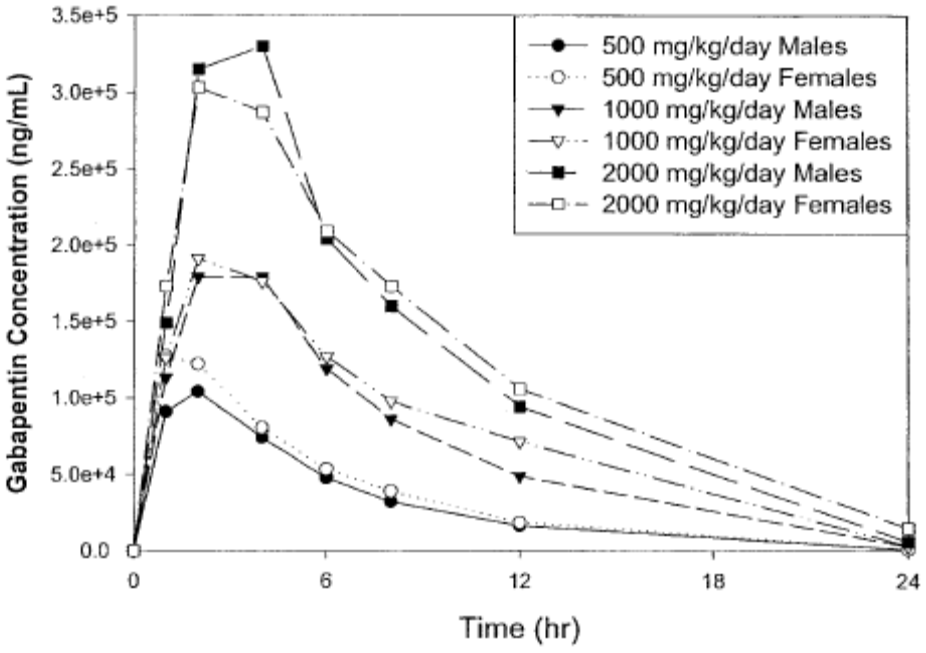
Table 4.3 Toxicokinetic Parameters for XP13512 in Blood of Monkeys After Oral Dosing of XP13512 – DAY 1

Group	Dose	Gender	N	C_{max} (µg/mL)	T_{max} (hr)	$T_{1/2}$ (hr)	AUC_{last} (µg*hr/mL)	$AUC_{(0-inf)}$ (µg*hr/mL)
2	500 mg/kg/day	F	4	4.77	1.00	2.02	8.39	8.51
		M	4	3.02	1.00	1.20	5.38	5.46
		Combined Ave.	8	3.89	1.00	1.61	6.89	6.98
3	1000 mg/kg/day	F	4	4.73	1.00	1.73	13.0	13.3
		M	4	5.89	1.50	2.25	17.5	17.8
		Combined Ave.	8	5.31	1.25	1.99	15.3	15.6
4	2000 mg/kg/day	F	4	14.8	1.75	3.95	55.6	64.2
		M	4	22.5	1.75	3.13	76.2	88.6
		Combined Ave.	8	18.7	1.75	3.54	65.9	76.4

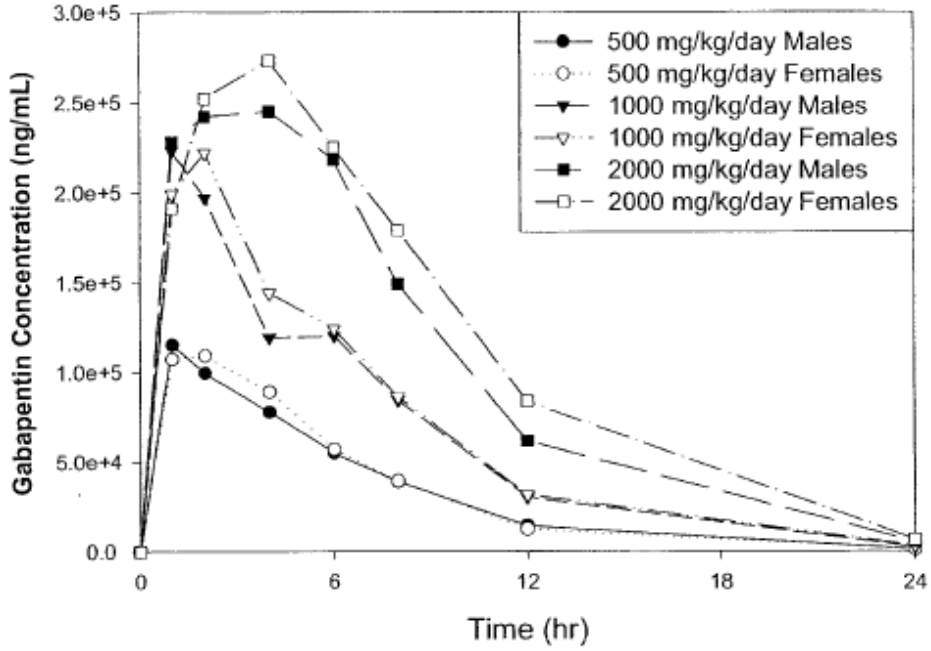
Table 4.4 Toxicokinetic Parameters for XP13512 in Blood of Monkeys After Oral Dosing of XP13512 – DAY 90

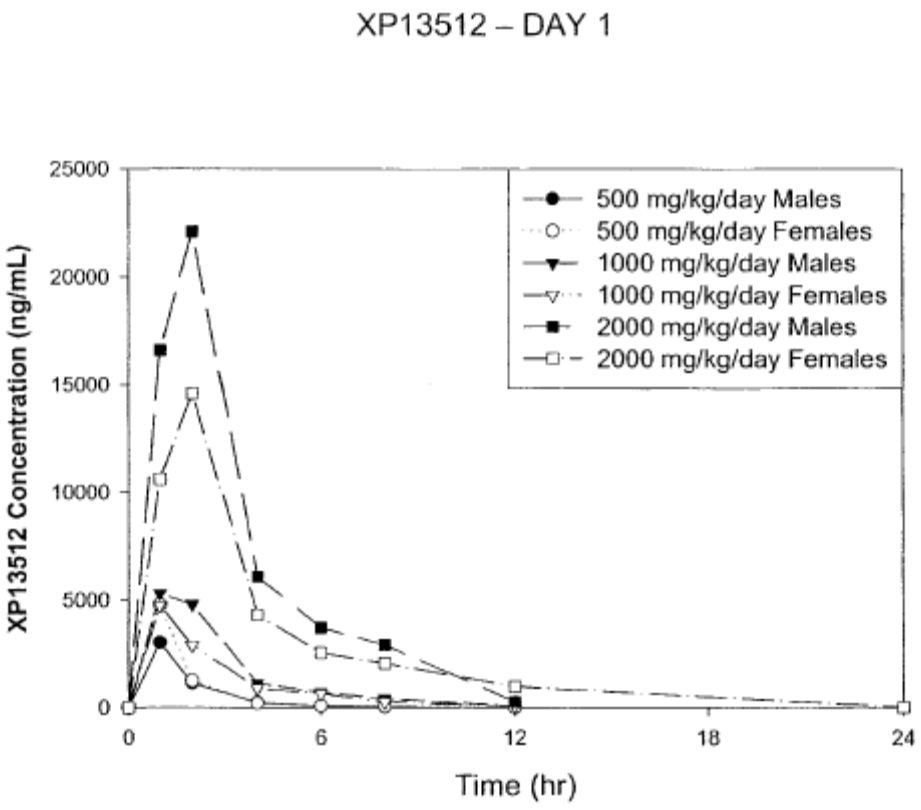
Group	Dose	Gender	N	C_{max} (µg/mL)	T_{max} (hr)	$T_{1/2}$ (hr)	AUC_{last} (µg*hr/mL)	$AUC_{(0-24)}$ (µg*hr/mL)
2	500 mg/kg/day	F	3	5.98	1.33	0.946	13.7	13.7
		M	4	4.49	1.00	1.00	8.62	8.62
		Combined Ave.	7	5.13	1.14	0.980	10.8	10.8
3	1000 mg/kg/day	F	4	11.0	1.00	1.01	23.6	23.6
		M	4	12.5	1.00	1.75	21.0	21.0
		Combined Ave.	8	11.8	1.00	1.38	22.3	22.3
4	2000 mg/kg/day	F	4	14.7	1.25	1.57	59.2	59.2
		M	3	16.9	1.00	1.59	48.0	48.0
		Combined Ave.	7	15.6	1.14	1.58	54.4	54.4

Gabapentin – DAY 1



Gabapentin – DAY 90





Study title: **9-Month Oral Toxicity Study of XP13512 in Monkeys with a 6-Month Interim Necropsy and a 1-Month Recovery Period**

Key study findings: Cynomolgus monkeys were treated by oral gavage with 0, 250, 1000 or 2000 mg/kg/d of XP13512. No adverse effects of treatment were found in any of the parameters evaluated. The NOEL is determined to be 2000 mg/kg/d. Exposures to gabapentin at the highest dose were 3370 µg.h/mL at the end of the 9 month period while exposures to XP13512 were 54.3 µg.h/mL at the same time point demonstrating extensive hydrolysis of the test article to gabapentin. The associated Cmax values were 366 µg/mL and 13.7 µg/mL, respectively. In the escalating dose study (Study RD2007/01520/00; doses: 1000, 2000, 3500 or 5000 mg/kg) in cynomolgus monkeys, adverse clinical signs at ≥1000 mg/kg included excessive salivation and vomition promptly after dosing thus obviating the animals absorbing the entire dose. The two-week study (RD2007/01521/00; doses: 0, 250, 750 or 2000 mg/kg/d) showed 2/3 of the high dose females vomited their dose on 7/14 days. While no other toxicities were shown at 2000 mg/kg/d in the 13-week or the 9-month studies, the high dose is consistent with OECD and ICH M3 R2 guidance. Therefore, this study is considered an adequate assessment of the toxicity of gabapentin encarbil in cynomolgus monkeys.

Study no.: 1032-032 or RD2007/01528/00XP047
Volume and page #: Electronic submission

Conducting laboratory and location: (b) (4)
Date of study initiation: 2/22/05
GLP compliance: Yes
QA report: yes
Drug, lot #, and % purity: Batch 12 at >99.0% purity

Methods

Doses: 0 (vehicle), 250, 1000 or 2000 mg/kg/d

Species/strain: Cynomolgus monkeys of Vietnamese origin

Number/sex/group or time point (main study): 6/sex/group for low and mid doses, 8/sex/group for control and high doses

Route, formulation, volume, and infusion rate: Oral gavage in 0.1% Tween 80® + 0.5% methylcellulose at 10 mL/kg

Satellite groups used for toxicokinetics or recovery: 2/sex/group were euthanized after 6 mos of dosing and 2/sex/control and high dose groups were maintained for a 1-month recovery period.

Age: 1 yr, 7 mos of age to 3 yrs, 4 mos of age

Weight: 1.67- 2.33 kg

Sampling times: TK samples were taken from 3/sex/group on Day1 and prior to necropsies at 0 (Day 1 only), 1, 2, 4, 6, 10, and 24 hrs post-dosing

Observations and times:

Mortality: Twice daily
Clinical signs: Twice daily
Body weights: Weekly
Food consumption: Daily checks for availability of feed and water and quantitative assessments weekly
Ophthalmoscopy: Prior to study initiation and prior to necropsies
EKG: As for ophthalmoscopy at pre-dosing and 1 hr post-dosing
Hematology: As for ophthalmoscopy
Clinical chemistry: As for ophthalmoscopy
Urinalysis: As for ophthalmoscopy
Gross pathology: All animals on study
Histopathology: Adequate Battery: yes
Peer review: Not specified

Results

Mortality: There were no premature decedents.

Clinical signs: There were no treatment-related adverse clinical signs reported.

Body weights: No adverse effects of treatment were found in body weights.

Food consumption: Consumption was comparable across groups.

Ophthalmoscopy: No treatment-engendered lesions were described.

EKG: No significant differences from baseline were found in any dose group.

Hematology: There were no biologically significant effects of dosing in any dose group.

Clinical chemistry: No dose-dependent differences were found between groups.

Urinalysis: No adverse effects on any urinary parameter were appreciated.

Gross pathology: No treatment-attributable gross lesions were described.

Organ weights: Organ weights were comparable across groups.

Histopathology: Adequate Battery: yes
Peer review: Not specified

There were no consistent adverse effects described in the histologic evaluation.

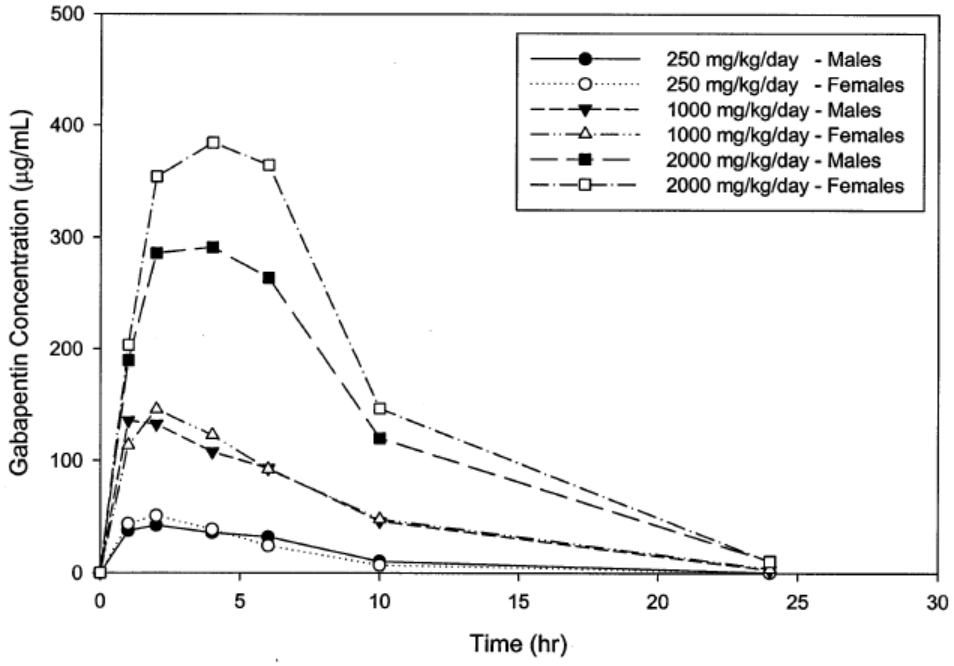
Toxicokinetics: Conversion of XP13512 to gabapentin was via hydrolysis and occurred rapidly. Exposures to gabapentin increased with increasing dose and no accumulation was evident.
Sponsor table:

Whole Blood Exposure of XP13512 and Gabapentin After Oral Administration of XP13512 in Monkeys in a 9-Month Toxicity Study

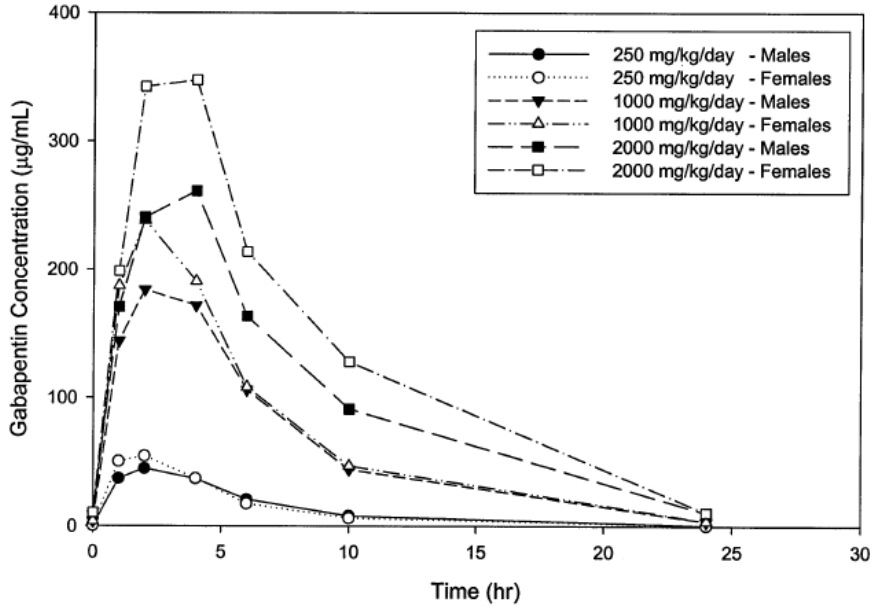
Parameter	XP13512 Dose (mg/kg)	Gabapentin			XP13512		
		Day 1	Month 6	Month 9	Day 1	Month 6	Month 9
AUC (μg·hr/mL)[a]	250	353	316	442	3.98	3.47	4.42
	1000	1310	1600	1740	26.9	32.6	33.4
	2000	3630	2860	3370	80.3	60.0	54.3
C_{max} (μg/mL)[a]	250	48.6	52.2	79.2	1.78	2.27	2.77
	1000	146	211	233	8.06	15.9	13.1
	2000	359	329	366	22.3	18.7	13.7
T_{max} (hr)[a]	250	2.50	1.83	1.50	1.00	1.00	1.00
	1000	2.00	2.00	2.25	1.00	1.00	1.25
	2000	4.00	3.33	3.17	1.83	1.33	1.83

[a]Abbreviations: AUC = Area under the concentration vs. time curve ($AUC_{(0\text{-}inf)}$ on Day 1 and $AUC_{(0\text{-}24)}$ at Months 6 and 9); C_{max} = Concentration maximum; T_{max} = Time to C_{max}.

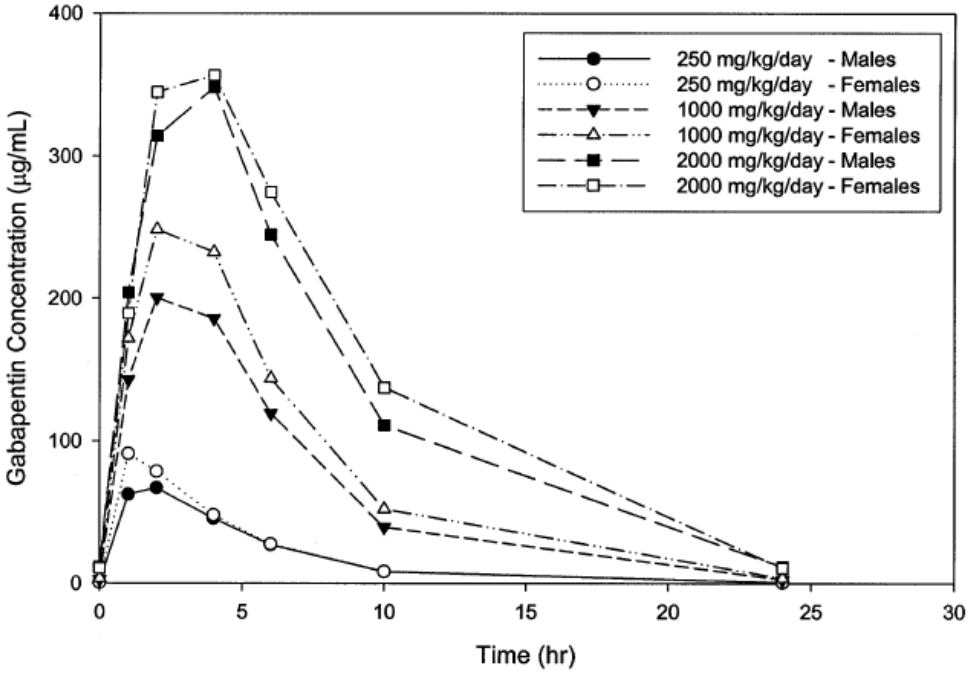
Gabapentin – DAY 1



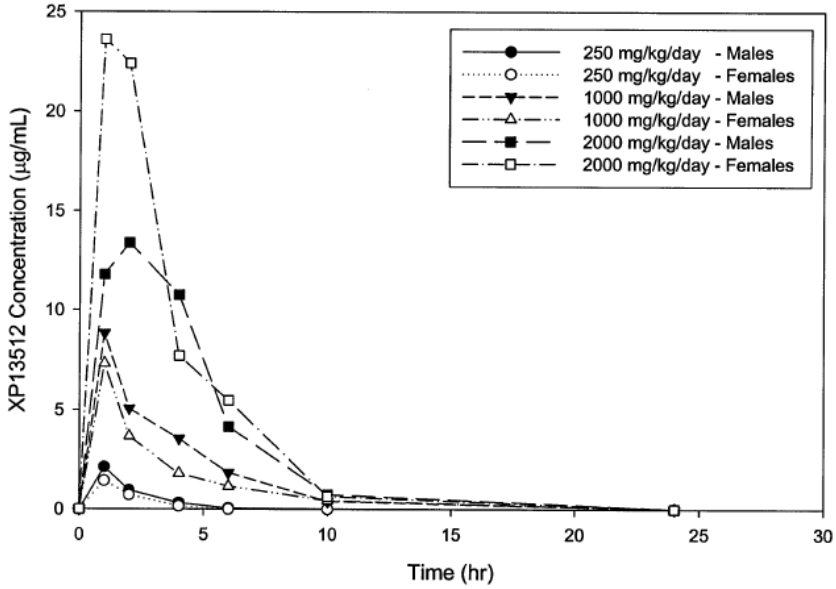
Gabapentin – 6 Months



Gabapentin – 9 Months



XP13512 – DAY 1



Sponsor table:

Appendix E (contd.). Toxicokinetic Parameters for XP13512 in Blood of Individual Monkeys After Oral Dosing of XP13512-9-Month

Group	Dose (mg/kg/day)	Drug	Gender	Animal	C_{max} (µg/mL)	T_{max} (hr)	$T_{1/2}$ (hr)	AUC_{last} (µg*hr/mL)	$AUC_{(0-24)}$ (µg*hr/mL)
1	0	XP13512	M	101	0.00	ND	ND	0.00	0.00
				102	0.00	ND	ND	0.00	0.00
				103	0.00	ND	ND	0.00	0.00
				129	0.00	ND	ND	0.00	0.00
				130	0.00	ND	ND	0.00	0.00
				131	0.00	ND	ND	0.00	0.00
				MEAN	0.00	ND	ND	0.00	0.00
				SD	0.00	ND	ND	0.00	0.00
			F	105	0.00	ND	ND	0.00	0.00
				106	0.00	ND	ND	0.00	0.00
				107	0.00	ND	ND	0.00	0.00
				133	0.00	ND	ND	0.00	0.00
				134	0.00	ND	ND	0.00	0.00
				135	0.00	ND	ND	0.00	0.00
				MEAN	0.00	ND	ND	0.00	0.00
				SD	0.00	ND	ND	0.00	0.00
			Combined	MEAN	0.00	ND	ND	0.00	0.00
				SD	0.00	ND	ND	0.00	0.00

ND – Not determined due to lack of quantifiable data points.

Appendix E (continued). Toxicokinetic Parameters for Gabapentin and XP13512 in Blood of Individual Monkeys After Oral Dosing of XP13512-9-Month

Group	Dose (mg/kg/day)	Drug	Gender	Animal	C_{max} (µg/mL)	T_{max} (hr)	$T_{1/2}$ (hr)	AUC_{last} (µg*hr/mL)	$AUC_{(0-24)}$ (µg*hr/mL)
2	250	Gabapentin	M	109	54.3	2.00	2.88	353	353
				110	99.6	2.00	3.77	552	552
				137	65.1	2.00	3.41	400	400
				138	50.5	2.00	3.25	362	362
				MEAN	67.4	2.00	3.33	417	417
				SD	22.4	0.00	0.369	92.5	92.5
			F	112	94.8	1.00	3.02	465	465
				113	69.4	1.00	3.15	384	384
				140	119	1.00	2.69	493	493
				141	81.0	1.00	3.50	529	529
				MEAN	91.1	1.00	3.09	468	468
				SD	21.3	0.00	0.334	61.5	61.5
			Combined	MEAN	79.2	1.50	3.21	442	442
				SD	23.9	0.535	0.350	77.7	77.7
Group	Dose (mg/kg/day)	Drug	Gender	Animal	C_{max} (µg/mL)	T_{max} (hr)	$T_{1/2}$ (hr)	AUC_{last} (µg*hr/mL)	$AUC_{(0-24)}$ (µg*hr/mL)
2	250	XP13512	M	109	1.08	1.00	1.09	2.27	2.44
				110	4.78	1.00	ND	6.03	8.52
				137	1.69	1.00	0.462	2.97	2.99
				138	1.71	1.00	1.33	4.11	4.29
				MEAN	2.32	1.00	0.960	3.84	4.56
				SD	1.67	0.00	0.447	1.64	2.75
			F	112	3.28	1.00	ND	3.32	3.40
				113	0.807	1.00	ND	0.937	1.20
				140	6.97	1.00	1.75	9.28	9.55
				141	1.82	1.00	0.566	2.89	2.93
				MEAN	3.22	1.00	1.16	4.11	4.27
				SD	2.70	0.00	0.837	3.60	3.64
			Combined	MEAN	2.77	1.00	1.04	3.98	4.42
				SD	2.13	0.00	0.536	2.59	2.99

ND – Not determined due to lack of quantifiable data points.

Appendix E (continued). Toxicokinetic Parameters for Gabapentin and XP13512 in Blood of Individual Monkeys After Oral Dosing of XP13512-9-Month

Group	Dose (mg/kg/day)	Drug	Gender	Animal	C_{max} (µg/mL)	T_{max} (hr)	$T_{1/2}$ (hr)	AUC_{last} (µg*hr/mL)	$AUC_{(0-24)}$ (µg*hr/mL)
3	1000	Gabapentin	M	115	189	2.00	3.13	1190	1190
				116	187	2.00	3.43	1430	1430
				143	225	2.00	3.23	1950	1950
				144	200	2.00	3.35	1620	1620
				MEAN	200	2.00	3.28	1550	1550
				SD	17.5	0.00	0.133	320	320
			F	118	235	2.00	3.32	1740	1740
				119	241	2.00	3.46	1560	1560
				146	322	4.00	3.33	2420	2420
				147	262	2.00	3.44	2030	2030
				MEAN	265	2.50	3.39	1940	1940
				SD	39.7	1.00	0.0737	375	375
			Combined	MEAN	233	2.25	3.34	1740	1740
				SD	44.8	0.707	0.113	385	385
Group	Dose (mg/kg/day)	Drug	Gender	Animal	C_{max} (µg/mL)	T_{max} (hr)	$T_{1/2}$ (hr)	AUC_{last} (µg*hr/mL)	$AUC_{(0-24)}$ (µg*hr/mL)
3	1000	XP13512	M	115	6.87	2.00	ND	17.3	18.6
				116	11.2	1.00	0.611	28.2	28.3
				143	15.3	1.00	1.05	26.7	26.9
				144	10.7	1.00	1.34	29.4	30.2
				MEAN	11.0	1.25	1.00	25.4	26.0
				SD	3.45	0.500	0.369	5.51	5.09
			F	118	18.7	1.00	0.683	37.2	37.4
				119	8.29	1.00	1.13	14.4	14.7
				146	21.8	2.00	0.888	85.2	85.7
				147	12.3	1.00	1.37	24.8	25.7
				MEAN	15.3	1.25	1.02	40.4	40.9
				SD	6.11	0.500	0.297	31.3	31.3
			Combined	MEAN	13.1	1.25	1.01	32.9	33.4
				SD	5.12	0.463	0.299	22.3	22.2

ND- Not determined due to lack of quantifiable data points.

Appendix E (continued). Toxicokinetic Parameters for Gabapentin and XP13512 in Blood of Individual Monkeys After Or Dosing of XP13512-9-Month

Group	Dose (mg/kg/day)	Drug	Gender	Animal	C_{max} (µg/mL)	T_{max} (hr)	$T_{1/2}$ (hr)	AUC_{last} (µg*hr/mL)	$AUC_{(0-24)}$ (µg*hr/mL)
4	2000	Gabapentin	M	121	440	4.00	3.43	3710	3710
				122	300	2.00	3.09	2110	2110
				123	339	4.00	4.21	2830	2830
				149	447	2.00	3.90	3860	3860
				150	369	4.00	4.70	3990	3990
				151	247	4.00	4.93	2640	2640
				MEAN	357	3.33	4.04	3190	3190
				SD	78.5	1.03	0.715	769	769
			F	125	349	4.00	3.49	3000	3000
				126	335	2.00	4.44	3190	3190
				127	468	2.00	3.43	4310	4310
				153	317	4.00	4.11	3590	3590
				154	420	4.00	3.38	4080	4080
				155	358	2.00	4.34	3170	3170
				MEAN	375	3.00	3.86	3560	3560
				SD	57.6	1.10	0.487	536	536
			Combined	MEAN	366	3.17	3.95	3370	3370
				SD	66.3	1.03	0.590	660	660

Appendix E (continued). Toxicokinetic Parameters for Gabapentin and XP13512 in Blood of Individual Monkeys After Oral Dosing of XP13512-9-Month

Group	Dose (mg/kg/day)	Drug	Gender	Animal	C_{max} (µg/mL)	T_{max} (hr)	$T_{1/2}$ (hr)	AUC_{last} (µg*hr/mL)	$AUC_{(0-24)}$ (µg*hr/mL)
4	2000	XP13512	M	121	13.0	2.00	0.908	44.4	44.6
				122	14.4	1.00	0.484	42.2	42.3
				123	5.40	1.00	1.77	30.3	33.6
				149	19.0	2.00	1.34	70.7	73.3
				150	10.8	4.00	3.41	47.9	47.9
				151	8.51	2.00	1.90	36.2	39.9
				MEAN	11.9	2.00	1.64	45.3	46.9
				SD	4.75	1.10	1.02	13.9	13.8
			F	125	7.73	1.00	3.61	47.5	56.6
				126	18.4	1.00	2.53	53.7	64.5
				127	18.1	1.00	1.25	57.0	58.1
				153	19.9	1.00	2.21	49.4	53.8
				154	14.3	4.00	1.43	65.4	70.0
				155	15.1	2.00	1.58	62.4	66.4
				MEAN	15.6	1.67	2.10	55.9	61.6
				SD	4.39	1.21	0.886	7.09	6.30
			Combined	MEAN	13.7	1.83	1.87	50.6	54.3
				SD	4.78	1.11	0.942	11.9	12.8

2.6.6.4 Genetic toxicology

Study title: **Chromosomal Aberrations in Cultured Human Peripheral Blood Lymphocytes**

Key findings: XP13512 induced chromosomal aberrations with and without S9 metabolic activation, but elicited neither endoreduplication nor polyploidy.

Study no.: RD2007/01489/00XP013 or 25183-0-449OECD or 7401-112

Volume and page #: Electronic submission
Conducting laboratory and location: (b) (4)
Date of study initiation: 6/11/03
GLP compliance: Yes
QA reports: yes
Drug, lot #, and % purity: XP13512, HD No. 1562/03-7401 HD Lot No. 2, Lot No 2892.A.03.2 at 97.3% purity

Methods
Strains/species/cell line: Human whole blood lymphocytes

Doses used in definitive study: 250, 500, 750, 1000, 1250, and 1500 µg/mL with S9; 250, 500, 1000, 1500, 1750 and 2000 µg/mL without S9 activation

Repeat assay with S9: 250, 500, 750, 1000, 1250 and 1500 µg/mL; no repeat assay performed without S9

Basis of dose selection: Initial assay with a maximal concentration of 3500 µg/mL (24-3500 µg/mL) using standard methodology; the 412, 588, 840 and 1200 µg/mL cultures with S9 were evaluated. A significant increase in aberrations was found at 1200 µg/mL, but neither polyploidy nor endoreduplication was seen. A repeat assay (250- 2000 µg/mL) was performed without S9 due to toxicity at 2450 µg/mL. Increased structural aberrations were noted at 1500- 2000 µg/mL; neither polyploidy nor endoreduplication was seen.

Negative controls: Culture medium and/or DMSO

Positive controls: Mitomycin C without S9, cyclophosphamide with S9

Incubation and sampling times: Standardized methodology

Results
Study validity: The positive and negative controls performed as anticipated so this study is considered adequate for regulatory purposes.

Study outcome: First assay: Without S9 activation, a test article precipitate was found at 2450 and 3500 µg/mL and hemolysis was also appreciated. Cytotoxicity was complete (essentially 100%) at 3500 µg/mL. Since a ≤2% decrease in mitotic index was found at 1720 µg/mL and a 99% reduction was found at 2450 µg/mL, the study was repeated. In the repeat assay, reductions of 0 17, 41, 57 and 100% were found at 1000, 1500, 1750, 2000 and 2500 µg/mL, respectively. A significant increase in aberrations was found at ≥1500 µg/mL, with no increases in endoreduplication or polyploidy.

First assay: With S9 activation, precipitation was found at ≥2450 µg/mL and hemolysis was appreciated. Cytotoxicity was complete at 3500 µg/mL. A 0% decrease (compared to controls) in mitotic index was found at 588 µg/mL, but a 52% decrease was seen at 1200 µg/mL and 81% at 1720 µg/mL and 99% reduction at 2450 µg/mL. Increased chromosomal aberrations were seen at 1200 µg/mL, but no increases in polyploidy or endoreduplication was found. In the confirmatory assay, reduced mitotic indices were found at 1250 µg/mL (30%) and 1500 µg/mL (45%) and significant increases in chromosomal aberrations were determined at ≥250 µg/mL, with no increases in endoreduplication or polyploidy.

Sponsor tables:

Table 3: Chromosome Aberrations in Human Lymphocytes - Without Metabolic Activation - approximately 3 Hour Treatment, approximately 22 Hour Harvest

Assay No.: 25183 Trial No.: B2 Date: 07/23/03 Lab No.: CY072203 Test Article: XP13512

				CELLS SCORED	% MITOTIC INDEX REDUCTION [a]	# ENDOREDUPLICATED CELLS	# POLYPLOID CELLS	JUDGEMENT (+/-) [b]	NUMBERS AND PERCENTAGES (%) OF CELLS SHOWING STRUCTURAL CHROMOSOME ABERRATIONS: Gaps	Simple Breaks	chte	chre	mab	TOTALS [c] -g	TOTALS [c] +g	JUDGEMENT (+/-) [d]
CONTROLS																
NEGATIVE:	RPMI 1640		A	100		0	0							0	0	
			B	100		0	0							0	0	
			TOTAL	200										0	0	
			AVERAGE	%	-	0.0	0.0							0.0	0.0	
VEHICLE:	DMSO	10.0µL/mL	A	100		0	0							0	0	
			B	100		0	0		1					0	1	
			TOTAL	200					1					0	1	
			AVERAGE	%	0	0.0	0.0		0.5					0.0	0.5	
POSITIVE:	MMC	1.00µg/mL	A	75		0	0		3	13	8			20	21	
			B	50		0	0		4	16	10			19	21	
			TOTAL	125					7	29	18			39	42	
			AVERAGE	%	-	0.0	0.0	-	5.6	23.2	14.4			31.2	33.6	+

chte: chromatid exchange chre: chromosome exchange mab: multiple aberrations, greater than 4 aberrations

a % Mitotic index reduction as compared to the vehicle control. b Significantly greater in % polyploidy and % endoreduplication than the vehicle control, $p \leq 0.01$. c -g = # or % of cells with chromosome aberrations; +g = # or % of cells with chromosome aberrations + # or % of cells with gaps. d Significantly greater in -g than the vehicle control, $p \leq 0.01$.

RPMI 1640= culture medium DMSO = Dimethylsulfoxide MMC = Mitomycin C

Xenoport Study No. XP013

Table 4: Chromosome Aberrations in Human Lymphocytes - Without Metabolic Activation - approximately 3 Hour Treatment, approximately 22 Hour Harvest

Assay No.: 25183 Trial No.: B2 Date: 07/23/03 Lab No.: CY072203 Test Article: XP13512

				CELLS SCORED	% MITOTIC INDEX REDUCTION [a]	# ENDOREDUPLICATED CELLS	# POLYPLOID CELLS	JUDGEMENT (+/-) [b]	NUMBERS AND PERCENTAGES (%) OF CELLS SHOWING STRUCTURAL CHROMOSOME ABERRATIONS: Gaps	Simple Breaks	chte	chre	mab	TOTALS [c] -g	TOTALS [c] +g	JUDGEMENT (+/-) [d]
TEST ARTICLE		250µg/mL	A	100		0	1		5	4				4	8	
			B	100		0	0		4	2				2	6	
			TOTAL	200					9	6				6	14	
			AVERAGE	%	-	0.0	0.5	-	4.5	3.0				3.0	7.0	-
		500µg/mL	A	100		0	0		1	2				2	3	
			B	100		0	0		2	4				4	6	
			TOTAL	200					3	6				6	9	
			AVERAGE	%	-	0.0	0.0	-	1.5	3.0				3.0	4.5	-
		1000µg/mL	A	100		0	0			1				1	1	
			B	100		0	0		1	1	1			2	3	
			TOTAL	200					1	2	1			3	4	
			AVERAGE	%	0	0.0	0.0	-	0.5	1.0	0.5			1.5	2.0	-
		1500µg/mL	A	75		0	0		1	9	1			9	10	
			B	100		0	0		4	18	1	1		19	22	
			TOTAL	175					5	27	2	1		28	32	
			AVERAGE	%	17	0.0	0.0	-	2.9	15.4	1.1	0.6		16.0	18.3	+
		1750µg/mL	A	100		0	0		2	16	1			16	18	
			B	100		0	0		3	13				13	14	
			TOTAL	200					5	29	1			29	32	
			AVERAGE	%	41	0.0	0.0	-	2.5	14.5	0.5			14.5	16.0	+
		2000µg/mL	A	75		0	0		2	11	2		1	14	16	
			B	50		0	0		3	13	2		1	14	17	
			TOTAL	125					5	24	4		2	28	33	
			AVERAGE	%	57	0.0	0.0	-	4.0	19.2	3.2		1.6	22.4	26.4	+

chte: chromatid exchange chre: chromosome exchange mab: multiple aberrations, greater than 4 aberrations

a % Mitotic index reduction as compared to the vehicle control. b Significantly greater in % polyploidy and % endoreduplication than the vehicle control, $p \leq 0.01$. c -g = # or % of cells with chromosome aberrations; +g = # or % of cells with chromosome aberrations + # or % of cells with gaps. d Significantly greater in -g than the vehicle control, $p \leq .01$.

RPMI 1640= culture medium DMSO = Dimethylsulfoxide MMC = Mitomycin C

Xenoport Study No: XP013

Table 6: Chromosome Aberrations in Human Lymphocytes -
With Metabolic Activation - approximately 3 Hour Treatment, approximately 22 Hour Harvest

Assay No.: 25183 Trial No.: B1 Date: 06/19/03 Lab No.: CY062703 Test Article: XP13512

				% MITOTIC INDEX REDUC-TION [a]	# ENDO-REDUPLI-CATED CELLS	# POLY-PLOID CELLS	JUDGE-MENT (+/-) [b]	NUMBERS AND PERCENTAGES (%) OF CELLS SHOWING STRUCTURAL CHROMOSOME ABERRATIONS							JUDGE-MENT (+/-) [d]
		CELLS SCORED						Gaps	Simple Breaks	chte	chre	mab	TOTALS [c] -g	TOTALS [c] +g	
CONTROLS															
NEGATIVE: RPMI 1640		A	100		0	0		1					0	1	
		B	100		0	0		3	1				1	4	
		TOTAL	200					4	1				1	5	
		AVERAGE	**%**	-	**0.0**	**0.0**		**2.0**	**0.5**				**0.5**	**2.5**	
VEHICLE: DMSO	10.0 µL/mL	A	100		0	0		1	1				1	2	
		B	100		0	0							0	0	
		TOTAL	200					1	1				1	2	
		AVERAGE	**%**	**0**	**0.0**	**0.0**		**0.5**	**0.5**				**0.5**	**1.0**	
POSITIVE: CP	25.0 µg/mL	A	50		0	0		8	19	4			20	25	
		B	50		0	0		4	16	5			18	20	
		TOTAL	100					12	35	9			38	45	
		AVERAGE	**%**	-	**0.0**	**0.0**	-	**12.0**	**35.0**	**9.0**			**38.0**	**45.0**	+
TEST ARTICLE	412 µg/mL	A	100		0	0							0	0	
		B	100		0	0		1					0	1	
		TOTAL	200					1					0	1	
		AVERAGE	**%**	**0**	**0.0**	**0.0**	-	**0.5**					**0.0**	**0.5**	-
	588 µg/mL	A	100		0	0		3	3				3	6	
		B	100		0	0		4	1				1	5	
		TOTAL	200					7	4				4	11	
		AVERAGE	**%**	**0**	**0.0**	**0.0**	-	**3.5**	**2.0**				**2.0**	**5.5**	-
	840 µg/mL	A	100		0	0		3	1				1	4	
		B	100		0	0		6	6				6	9	
		TOTAL	200					9	7				7	13	
		AVERAGE	**%**	**5**	**0.0**	**0.0**	-	**4.5**	**3.5**				**3.5**	**6.5**	-
	1200 µg/mL	A	50		0	0		5	16	1			17	20	
		B	50		0	0		5	12	4			16	19	
		TOTAL	100					10	28	5			33	39	
		AVERAGE	**%**	**52**	**0.0**	**0.0**	-	**10.0**	**28.0**	**5.0**			**33.0**	**39.0**	+

chte: chromatid exchange chre: chromosome exchange mab: multiple aberrations, greater than 4 aberrations

[a] % Mitotic index reduction as compared to the vehicle control.

[b] Significantly greater in % polyploidy and % endoreduplication than the vehicle control, $p \leq 0.01$.

[c] -g = # or % of cells with chromosome aberrations; +g = # or % of cells with chromosome aberrations + # or % of cells with gaps.

[d] Significantly greater in -g than the vehicle control, p. 0.01. RPMI 1640 = culture medium DMSO = Dimethylsulfoxide CP = Cyclophosphamide

Table 8: Chromosome Aberrations in Human Lymphocytes - With Metabolic Activation - approximately 3 Hour Treatment, approximately 22 Hour Harvest

Assay No.: 25183 Trial No.: C1 Date: 07/23/03 Lab No.: CY072203 Test Article: XP13512

			CELLS SCORED	% MITOTIC INDEX REDUCTION [a]	# ENDOREDUPLICATED CELLS	# POLYPLOID CELLS	JUDGEMENT (+/-) [b]	NUMBERS AND PERCENTAGES (%) OF CELLS SHOWING STRUCTURAL CHROMOSOME ABERRATIONS: Gaps	Simple Breaks	chte	chre	mab	TOTALS [c] -g	TOTALS [c] +g	JUDGEMENT (+/-) [d]
CONTROLS															
NEGATIVE: RPMI 1640		A	100		0	0		1					0	1	
		B	100		0	0		1					0	1	
		TOTAL	200					2					0	2	
		AVERAGE	%	-	0.0	0.0		1.0					0.0	1.0	
VEHICLE: DMSO	10.0µL/mL	A	100		0	0			1				1	1	
		B	100		0	0							0	0	
		TOTAL	200						1				1	1	
		AVERAGE	%	0	0.0	0.0			0.5				0.5	0.5	
POSITIVE: CP	25.0µg/mL	A	75		0	0		3	25	2		1	28	29	
		B	50		0	0		2	18	2		1	19	20	
		TOTAL	125					5	43	4		2	47	49	
		AVERAGE	%	-	0.0	0.0	-	4.0	34.4	3.2		1.6	37.6	39.2	+
TEST ARTICLE	250µg/mL	A	100		0	0		3	3				3	6	
		B	100		0	0		6	2				2	8	
		TOTAL	200					9	5				5	14	
		AVERAGE	%	-	0.0	0.0	-	4.5	2.5				2.5	7.0	-
	500µg/mL	A	100		0	1		7	10				10	15	
		B	100		0	0		7	10	1			10	16	
		TOTAL	200					14	20	1			20	31	
		AVERAGE	%	-	0.0	0.5	-	7.0	10.0	0.5			10.0	15.5	+
	750µg/mL	A	75		0	0		3	16	1			17	20	
		B	75		0	0		1	12	2			13	14	
		TOTAL	150					4	28	3			30	34	
		AVERAGE	%	-	0.0	0.0	-	2.7	18.7	2.0			20.0	22.7	+
	1000µg/mL	A	50		0	0		1	21				21	22	
		B	50		0	0		2	13	3			15	16	
		TOTAL	100					3	34	3			36	38	
		AVERAGE	%	0	0.0	0.0	-	3.0	34.0	3.0			36.0	38.0	+
	1250µg/mL	A	50		0	0		4	18	3			19	23	
		B	50		0	0		2	19	4		1	23	25	
		TOTAL	100					6	37	7		1	42	48	
		AVERAGE	%	30	0.0	0.0	-	6.0	37.0	7.0		1.0	42.0	48.0	+
	1500µg/mL	A	50		0	0			19	4			21	21	
		B	50		0	0		1	19	4			21	22	
		TOTAL	100					1	38	8			42	43	
		AVERAGE	%	45	0.0	0.0	-	1.0	38.0	8.0			42.0	43.0	+

chte: chromatid exchange chre: chromosome exchange mab: multiple aberrations, greater than 4 aberrations

[a] % Mitotic index reduction as compared to the vehicle control. [b] Significantly greater in % polyploidy and % endoreduplication than the vehicle control, $p \leq 0.01$. [c] -g = # or % of cells with chromosome aberrations; +g = # or % of cells with chromosome aberrations + # or % of cells with gaps.

[d] Significantly greater in -g than the vehicle control, $p \leq 0.01$. RPMI 1640 = culture medium DMSO = Dimethylsulfoxide CP = Cyclophosphamide

The sponsor conducted Study #RD2008/00754/00XP020 (also PK-2003-016) entitled "Stability of XP13512 Under the Conditions of the *In Vitro* Human Lymphocyte Chromosomal Aberration Assay" to explain the positive chromosomal aberration findings. Human whole blood preparations were handled identically as in the experiment described above at doses of 1500 µM and 4500 µM with and without S9. When the prodrug was hydrolyzed, significant amounts of acetaldehyde were released (1500 µM dose: 608 µM without S9, 1150 µM with S9; 4500 µM dose: 901 µM without S9, 2730 µM with S9). Acetaldehyde concentrations were measured after the (b) (4) of 3 hrs. A review of the literature provided information that acetaldehyde at ≥100 µM can induce chromosomal aberrations in cultured cells. Therefore, this appears to be a reasonable explanation for this *in vitro* finding. However, the sponsor did not do controlled experiments to document this phenomenon with XP13512. *In vivo*, aldehyde dehydrogenase rapidly oxidizes acetaldehyde.

Study title: **Salmonella- Escherichia Coli/Mammalian-Microsome Reverse Mutation Assay**

Key findings: XP13512 did not elicit mutagenicity in any of the bacterial strains tested.

Study no.: 7401-122 or XP048 or RD2007/01490/00XP049

Volume and page #: Electronic submission
Conducting laboratory and location: (b) (4)
Date of study initiation: 1/27/05
GLP compliance: Yes
QA reports: yes
Drug, lot #, and % purity: Lot XN-001-10 at 99.9% purity

Methods
Strains/species/cell line: *Salmonella typhimurium* strains TA98, TA100, TA1535, TA1537; E. coli strain WP2*uvr*A

Doses used in definitive study: 33.3, 100, 333, 1000, 3330, and 5000 µg/plate with or without S9; commercial S9 from male Sprague-Dawley rats was used.

Basis of dose selection: Dose-range finding assays (#7401-113 and 7401-120; data provided) at 6.67, 10.0, 33.3, 66.7, 100, 333, 667, 1000, 3330, and 5000 µg/plate with no significant increase in revertants found at any dose with or without S9 added

Negative controls: DMSO

Positive controls:

Table I. Positive Controls

Tester Strain	S9 Mix	Positive Control	Dose (µg/plate)
TA98	+	benzo[a]pyrene	2.5
TA98	–	2-nitrofluorene	1.0
TA100	+	2-aminoanthracene	2.5
TA100	–	sodium azide	2.0
TA1535	+	2-aminoanthracene	2.5
TA1535	–	sodium azide	2.0
TA1537	+	2-aminoanthracene	2.5
TA1537	–	ICR-191	2.0
WP2*uvr*A	+	2-aminoanthracene	25.0
WP2*uvr*A	–	4-nitroquinoline-N-oxide	1.0

Incubation and sampling times: Standardized methodology

Results
Study validity: The positive and negative controls performed as anticipated so this study is considered adequate for regulatory purposes.

Study outcome: No significant increases in revertants were found at any dose of XP13512.
Sponsor tables:

	Dose/Plate	Mean Revertants Per Plate with Standard Deviation										Back-ground Lawn[a]
		TA98		TA100		TA1535		TA1537		WP2*uvr*A		
		Mean	S.D.	Mean	S.D.	Mean	S.D.	Mean	S.D.	Mean	S.D.	
Microsomes: Rat Liver												
Vehicle Control		21	4	98	9	14	1	9	3	21	6	N
Test Article	33.3 µg	22	5	98	3	11	8	5	3	19	5	N
	100 µg	29	2	103	15	18	3	6	3	16	5	N
	333 µg	27	11	99	6	18	7	11	5	15	5	N
	1000 µg	23	1	94	2	16	1	9	1	20	4	N
	3330 µg	22	4	103	11	19	2	9	1	21	5	N
	5000 µg	27	0	105	5	17	2	8	1	23	6	N
Positive Control[b]		390	55	1890	1201	140	21	142	20	623	108	N
Microsomes: None												
Vehicle Control		17	3	88	9	13	5	6	4	17	7	N
Test Article	33.3 µg	13	3	93	12	15	8	4	1	12	3	N
	100 µg	18	5	79	8	16	3	3	2	15	1	N
	333 µg	10	5	87	5	14	4	8	2	12	3	N
	1000 µg	14	1	85	18	14	3	6	4	16	5	N
	3330 µg	16	2	83	16	18	3	7	2	19	2	N
	5000 µg	15	2	81	13	12	2	5	2	16	5	N
Positive Control[c]		421	29	1479	61	1186	49	1016	148	464	70	N

[a] Background Lawn Evaluation Codes:
N = normal R = reduced O = obscured A = absent P = precipitate

[b]

TA98	benzo[a]pyrene	2.5 µg/plate
TA100	2-aminoanthracene	2.5 µg/plate
TA1535	2-aminoanthracene	2.5 µg/plate
TA1537	2-aminoanthracene	2.5 µg/plate
WP2*uvr*A	2-aminoanthracene	25.0 µg/plate

[c]

TA98	2-nitrofluorene	1.0 µg/plate
TA100	sodium azide	2.0 µg/plate
TA1535	sodium azide	2.0 µg/plate
TA1537	ICR-191	2.0 µg/plate
WP2*uvr*A	4-nitroquinoline-N-oxide	1.0 µg/plate

Study title: **Salmonella- Escherichia Coli/ Mammalian Microsome Reverse Mutation Assay**

Key findings: Under the conditions of this study, XP13512 at doses up to 5000 µg/plate did not elicit an increased number of revertants with or without metabolic activation. Thus XP13512 is not considered a mutagen in this study.

Study no.: RD2007/01491/00 or XP029 or 7401-120

Volume and page #: Electronic submission
Conducting laboratory and location: (b) (4)

Date of study initiation: 8/19/04
GLP compliance: Yes
QA reports: yes
Drug, lot #, and % purity: XN-001-3 at 99.5% purity

Methods
Strains/species/cell line: *E. coli* TA98, TA100, TA1535 and TA1537 and *S. typhimurium* WP2*uvr*A

Doses used in definitive study: 33.3, 100, 333, 1000, 3330, and 5000 µg/plate in triplicate.

Basis of dose selection: Range-finding assay at 6.67- 5000 µg/plate in strains TA100 and WP2*uvr*A

Negative controls: DMSO (vehicle)

Positive controls: Benzo[a]pyrene, 2-nitrofluorene, 2-aminoanthracene, sodium azide, 2-aminoacridine, ICR-191, and 4-nitroquinoline-N-oxide

Incubation and sampling times: Standardized methodology using commercial S9 from male Sprague-Dawley rats

Results
Study validity: The positive and negative controls performed as anticipated so this study is considered valid for regulatory purposes.

Study outcome: No cytotoxicity was appreciated at any dose of XP13512. No increases in the mean number of revertants were found at any dose, with or without S9 metabolic activation. Thus, the test article is not considered a mutagen under the conditions of this study.

Sponsor table:

	Dose/Plate	Mean Revertants Per Plate with Standard Deviation										Back-ground Lawn[a]
		TA98		TA100		TA1535		TA1537		WP2*uvr*A		
		Mean	S.D.	Mean	S.D.	Mean	S.D.	Mean	S.D.	Mean	S.D.	
Microsomes: Rat Liver												
Vehicle Control		21	6	93	8	13	2	7	2	15	6	N
Test Article	33.3 µg	22	2	106	20	12	1	8	5	21	2	N
	100 µg	22	4	97	2	13	4	4	1	21	1	N
	333 µg	18	8	94	18	10	5	10	1	23	4	N
	1000 µg	16	1	91	23	8	3	6	2	20	4	N
	3330 µg	28	3	89	14	14	4	5	2	16	2	N
	5000 µg	21	5	94	9	11	2	7	5	21	4	N
Positive Control[b]		412	43	1093	99	150	9	191	20	443	46	N
Microsomes: None												
Vehicle Control		10	2	96	12	14	5	6	0	12	6	N
Test Article	33.3 µg	16	5	89	17	14	3	5	1	16	3	N
	100 µg	11	1	81	14	9	1	2	2	18	3	N
	333 µg	13	4	83	4	11	5	6	2	14	5	N
	1000 µg	14	3	86	10	11	4	5	2	17	2	N
	3330 µg	13	2	98	10	9	1	5	2	19	1	N
	5000 µg	15	4	86	12	8	3	3	1	18	4	N
Positive Control[c]		258	38	1196	142	877	38	713	14	272	23	N

[a] Background Lawn Evaluation Codes:
N = normal R = reduced O = obscured A = absent P = precipitate

[b] TA98	benzo[a]pyrene	2.5 µg/plate	[c] TA98	2-nitrofluorene	1.0 µg/plate
TA100	2-aminoanthracene	2.5 µg/plate	TA100	sodium azide	2.0 µg/plate
TA1535	2-aminoanthracene	2.5 µg/plate	TA1535	sodium azide	2.0 µg/plate
TA1537	2-aminoanthracene	2.5 µg/plate	TA1537	ICR-191	2.0 µg/plate
WP2*uvr*A	2-aminoanthracene	25.0 µg/plate	WP2*uvr*A	4-nitroquinoline-N-oxide	1.0 µg/plate

Study title: **Salmonella- Escherichia Coli/ Mammalian Microsome Reverse Mutation Assay with A Confirmatory Assay**

Key findings: Under the conditions of this study, XP13512 at doses up to 5000 µg/plate did not elicit an increased number of revertants with or without metabolic activation and is not considered a mutagen in this study.

Study no.: RD2007/01492/00 or XP014 or 7401-113

Volume and page #: Electronic submission
Conducting laboratory and location: (b) (4)
Date of study initiation: 6/17/03
GLP compliance: Yes

QA reports: yes
Drug, lot #, and % purity: Lot 2892.A.03.2 at 97.3% purity

Methods
Strains/species/cell line: *E. coli* TA98, TA100, TA1535 and TA1537 and *S. typhimurium* WP2*uvr*A

Doses used in definitive study: 33.3, 100, 333, 1000, 3330, and 5000 µg/plate in triplicate.

Basis of dose selection: Range-finding assay at 6.67- 5000 µg/plate in strains TA100 and WP2*uvr*A

Negative controls: DMSO (vehicle)

Positive controls: Benzo[a]pyrene, 2-nitrofluorene, 2-aminoanthracene, sodium azide, 2-aminoacridine, ICR-191, and 4-nitroquinoline-N-oxide

Incubation and sampling times: Standardized methodology with commercial S9 from male Sprague-Dawley rats was used.

Results
Study validity: The positive and negative controls performed as expected. This study is considered valid for regulatory purposes.

Study outcome: No cytotoxicity was appreciated at any dose of XP13512. No increases in the mean number of revertants were found at any dose, with or without S9 metabolic activation. Thus, the test article is not considered a mutagen under the conditions of this study.

Study title: **In vivo Rat Micronucleus Assay with XP13512**

Key findings: There were no significant increases in micronucleated PCEs at any dose or time point evaluated. XP13512 is not a clastogen under the conditions of this study.

Study no.: RD2007/01488/00XP015 or 7401-116

Volume and page #: Electronic submission
Conducting laboratory and location: (b) (4)
Date of study initiation: 7/1/03
GLP compliance: Yes
QA reports: yes
Drug, lot #, and % purity: Lot 2892.A.03.2 at 97.3% purity

Methods

Strains/species/cell line: Male Crl:(WI)BR-Wistar rats with 5/group (including positive control) sacrificed at 24 hrs post-dosing, and 5 from high and vehicle control groups at 48 hrs post-dosing.

Doses used in definitive study: 0 (0.5% methycellulose/0.1% Tween 80), 500, 1000 or 2000 mg/kg

Basis of dose selection: Previously conducted studies in rats

Negative controls: Vehicle

Positive controls: Cyclophosphamide at 60 mg/kg p.o.

Incubation and sampling times: Standardized methodology

Results

Study validity: The positive and negative controls performed as anticipated. This study is considered adequate for regulatory purposes.

Study outcome: The mid and high dose animals demonstrated increased salivation immediately post-dosing but no other adverse effects were described.

No significant increases in micronucleated PCEs or decreases in the PCE:NCE ratios were found when the bone marrows were examined.

Sponsor table:

Table 1: Micronucleus Data Summary Table

Assay No.: 25183
Test Article: XP13512
Date Dosed: 01 July 2003

Treatment	Dose	Harvest Time	% Micronucleated PCEs Mean of 2000 per Animal ± S.E. Males	Ratio PCE:NCE Mean ± S.E. Males
Controls				
Vehicle	Vehicle	24 hr	0.03 ± 0.02	0.83 ± 0.07
		48 hr	0.06 ± 0.02	0.81 ± 0.11
Positive	CP 60mg/kg	24 hr	1.50 ± 0.05*	0.70 ± 0.06
Test Article	500mg/kg	24 hr	0.06 ± 0.02	1.15 ± 0.11
	1000mg/kg	24 hr	0.06 ± 0.03	1.28 ± 0.05
	2000mg/kg	24 hr	0.02 ± 0.02	0.90 ± 0.05
		48 hr	0.03 ± 0.01	1.04 ± 0.19

* Significantly greater than the corresponding vehicle control, $p \leq 0.01$.
Vehicle = 0.5% methylcellulose and 0.1% Tween 80 in reverse osmosis water
CP = Cyclophosphamide
PCE = Polychromatic erythrocyte
NCE = Normochromatic erythrocyte

Study title: **In vivo/in vitro Unscheduled DNA Synthesis in Rat Primary Hepatocyte Cultures at Two Timepoints**

Key findings: Under the conditions of this study, XP13512 is considered negative for UDS in treated male SD rats at doses ≤2000 mg/kg.

Study no.: RD2008/00149/00XP062 or 7401-123

Volume and page #: Electronic submission
Conducting laboratory and location: (b) (4)
Date of study initiation: 9/21/05
GLP compliance: Yes
QA reports: yes
Drug, lot #, and % purity: Lot #10 at ≥99.0% purity

Methods

Strains/species/cell line: Male Hsd:SD rats' hepatocytes (N=4 treated rats/ time point but UDS determined from 3/group) harvested at 2-4 hrs post-dosing and 14-16 hrs post-dosing

Doses used in definitive study: 0 (0.5% methylcellulose, 0.1% Tween 80), 500, 1000 or 2000 mg/kg in 10 mL/kg

Basis of dose selection: Not stated

Negative controls: Vehicle

Positive controls: N-dimethylnitrosamine (DMN) at 10 mg/kg (1 mL/kg i.p.) for the 2-4 hr harvest and 15 mg/kg for the 14-16 hr harvest

Incubation and sampling times: Standardized methodology

Results

Study validity: The positive and negative controls performed as anticipated so this study is considered adequate for regulatory purposes.

Study outcome: Slight hypoactivity just prior to perfusion was noted in one 2000 mg/kg animal (2-4 hr time point) but no other animals were similarly affected.

Sponsor table:

TABLE 1. SUMMARY OF UDS SLIDE DATA

Treatment	Dose (mg/kg)	N[a]	Time (hr)		Mean Nuclear Grains[b] ± SD	Mean Net Nuclear Grains[c] ± SD	Mean Cytoplasmic Grains[d] ± SD	Mean % Cells with ≥5 NNG[e] ± SD
Vehicle Control								
	0	3	2-4	Mean	1.68	-0.72	2.40	0.00
				± SD	0.44	0.36	0.19	0.00
	0	3	14-16	Mean	1.64	-0.40	2.04	0.22
				± SD	0.85	0.86	0.21	0.39
Positive Control								
	10	3	2-4	Mean	13.40	11.18	2.22	95.11
				± SD	2.08	1.60	0.84	4.73
	15	3	14-16	Mean	9.77	8.12	1.65	78.45
				± SD	1.67	1.51	0.37	9.10
Test Article								
	500	3	2-4	Mean	1.61	-0.84	2.45	0.00
				± SD	0.58	0.19	0.47	0.00
		3	14-16	Mean	1.92	-0.54	2.46	0.22
				± SD	0.24	0.23	0.21	0.39
	1000	3	2-4	Mean	1.59	-0.62	2.21	0.22
				± SD	0.40	0.27	0.23	0.39
		3	14-16	Mean	2.36	-0.01	2.37	0.44
				± SD	0.42	0.19	0.32	0.38
	2000	3	2-4	Mean	1.60	-0.73	2.33	0.00
				± SD	0.49	0.21	0.50	0.00
		3	14-16	Mean	1.93	-0.27	2.21	0.00
				± SD	0.37	0.33	0.20	0.00

Notes:
[a] Three animals per dose level were analyzed.
[b] Average nuclear grain count.
[c] Average of net nuclear grain count with standard deviation (SD) between coverslips.
Net nuclear grains (NNG) = Nuclear grain count - Average cytoplasmic grain count.
[d] Average of cytoplasmic grain count.
[e] Average percentage of cells with greater than or equal to 5 net nuclear grains.
Vehicle control article = 0.5% methylcellulose (medium viscosity, 1500 cps, w/v) and 0.1% Tween 80 (v/v) in reverse osmosis water, 10 mL/kg.
Positive control article = Dimethylnitrosamine, 1 mL/kg.
Test Article = XP13512, 10 mL/kg.
Criteria for a positive response:
2-4 hr timepoint – mean net nuclear grain counts ≥ 2.28 or nuclei containing ≥ 5 NNG ≥ 10.00%.
14-16 hr timepoint - mean net nuclear grain counts ≥ 2.60 or nuclei containing ≥ 5 NNG ≥ 10.22%.

Genotoxic Impurities:

Study title: (b) (4) **Reverse Mutation Assay "Ames Test" Using Salmonella Typhimurium and Escherichia Coli**

Key findings: Under the conditions of this study, (b) (4) impurity, is not considered a mutagen.

Study no.: 0102/0556 or ED2008/00053/00
Volume and page #: Electronic submission
Conducting laboratory and location: (b) (4)
Date of study initiation: 8/1/07
GLP compliance: OECD
QA reports: yes
Drug, lot #, and % purity: Batch 3107 purity not specified; test article also known at (b) (4) impurity.

Methods
Strains/species/cell line: *E. coli* TA98, TA100, TA1535 and TA1537 and *S. typhimurium* WP2*uvr*A

Doses used in definitive study: First assay: 0.5, 1.5, 5, 15, 50, 150, 500 and 1500 µg/plate in triplicate. Second assay: 0.5, 1.5, 5, 15, 50, 150 and 500 µg/plate

Basis of dose selection: Range-finding assay at 0, 0.15, 0.5, 1.5, 5, 15, 50, 150, 500, 1500 and 5000 µg/plate in strains TA100 and WP2*uvr*A. The bacterial lawns were markedly reduced at concentrations ≥500 µg/plate.

Negative controls: Acetone (vehicle)

Positive controls: N-ethyl-N'-nitro-N-nitrosoguanidine, 9-aminoacridine, 4-nitroquinoline-1-oxide, 2-aminoanthracene, benzo[a]pyrene

Incubation and sampling times: Standardized methodology with commercial S9 from male Sprague-Dawley rats was used.

Results
Study validity: The positive and negative controls performed as anticipated so this study is considered adequate for regulatory purposes.

Study outcome: Significant cytotoxicity was found in all strains tested with 500 µg/plate. No precipitates were seen. No increases in revertants were found at any concentration, with or without S9 metabolic activation. Under the conditions of this study, (b) (4) is not considered a mutagen.
Sponsor tables:

First assay:

Table 2 Test Results: Experiment 1 – Without Metabolic Activation

Test Period		From: 01 September 2007						To: 04 September 2007			
With or without S9-Mix	Test substance concentration (µg/plate)	Number of revertants (mean number of colonies per plate)									
		Base-pair substitution type						Frameshift type			
		TA100		TA1535		WP2uvrA-pKM101		TA98		TA1537	
-	0	95 66 91	(84) 15.7#	22 15 11	(16) 5.6	120 119 119	(119) 0.6	32 35 22	(30) 6.8	14 8 7	(10) 3.8
-	0.5	90 82 80	(84) 5.3	15 12 26	(18) 7.4	108 97 103	(103) 5.5	22 22 23	(22) 0.6	4 4 4	(4) 0.0
-	1.5	62 80 80	(74) 10.4	19 20 15	(18) 2.6	101 101 102	(101) 0.6	27 27 20	(25) 4.0	7 9 8	(8) 1.0
-	5	85 81 79	(82) 3.1	20 16 19	(18) 2.1	112 90 91	(98) 12.4	18 43 15	(25) 15.4	10 10 4	(8) 3.5
-	15	110 86 73	(90) 18.8	26 23 10	(20) 8.5	104 96 98	(99) 4.2	16 26 27	(23) 6.1	13 11 11	(12) 1.2
-	50	109 99 106	(105) 5.1	16 16 23	(18) 4.0	109 106 101	(105) 4.0	27 27 27	(27) 0.0	14 11 5	(10) 4.6
-	150	38 S 46 S 46 S	(43) 4.6	19 V 14 V 9 V	(14) 5.0	85 S 63 S 69 S	(72) 11.4	11 V 20 V 15 V	(15) 4.5	2 V 1 V 3 V	(2) 1.0
-	500	0 V 0 V 0 V	(0) 0.0	0 T 0 T 0 T	(0) 0.0	0 V 0 V 0 V	(0) 0.0	0 T 0 T 0 T	(0) 0.0	0 T 0 T 0 T	(0) 0.0
Positive controls	Name	ENNG		ENNG		ENNG		4NQO		9AA	
	Concentration (µg/plate)	3		5		0.5		0.2		80	
S9-Mix	No. colonies per plate	284 357 401	(347) 59.1	115 101 117	(111) 8.7	833 803 657	(764) 94.2	180 148 147	(158) 18.8	861 474 933	(756) 246.9
-											

Table 3 Test Results: Experiment 1 – With Metabolic Activation

Test Period		From: 01 September 2007						To: 04 September 2007					
With or without S9-Mix	Test substance concentration (µg/plate)	Number of revertants (mean number of colonies per plate)											
		Base-pair substitution type								Frameshift type			
		TA100		TA1535		WP2uvrA-pKM101		TA98		TA1537			
+	0	99 99 95	(98) 2.3#	7 12 19	(13) 6.0	135 144 134	(138) 5.5	27 46 22	(32) 12.7	7 11 14	(11) 3.5		
+	1.5	69 107 103	(93) 20.9	7 12 13	(11) 3.2	125 126 124	(125) 1.0	29 29 29	(29) 0.0	7 12 12	(10) 2.9		
+	5	95 99 98	(97) 2.1	12 14 16	(14) 2.0	159 103 133	(132) 28.0	18 16 36	(23) 11.0	7 8 8	(8) 0.6		
+	15	90 92 92	(91) 1.2	19 7 7	(11) 6.9	142 141 124	(136) 10.1	41 35 32	(36) 4.6	9 9 9	(9) 0.0		
+	50	103 104 91	(99) 7.2	20 19 20	(20) 0.6	136 136 131	(134) 2.9	24 25 38	(29) 7.8	4 4 4	(4) 0.0		
+	150	82 82 86	(83) 2.3	14 10 5	(10) 4.5	96 85 82	(88) 7.4	25 25 19	(23) 3.5	8 13 10	(10) 2.5		
+	500	38 V 36 V 33 V	(36) 2.5	12 V 2 V 4 V	(6) 5.3	22 V 34 V 23 V	(26) 6.7	10 V 10 V 8 V	(9) 1.2	5 S 10 S 9 S	(8) 2.6		
+	1500	0 T 0 T 0 T	(0) 0.0	0 T 0 T 0 T	(0) 0.0	0 T 0 T 0 T	(0) 0.0	0 T 0 T 0 T	(0) 0.0	0 T 0 T 0 T	(0) 0.0		
Positive controls	Name	2AA		2AA		2AA		BP		2AA			
	Concentration (µg/plate)	1		2		2		5		2			
S9-Mix	No. colonies per plate												
+		559 600 683	(614) 63.2	357 251 234	(281) 66.7	580 697 630	(636) 58.7	437 476 420	(444) 28.7	181 240 164	(195) 39.9		

Second assay:

Table 4 Test Results: Experiment 2 – Without Metabolic Activation

Test Period		From: 06 September 2007						To: 09 September 2007			
With or without S9-Mix	Test substance concentration (µg/plate)	Number of revertants (mean number of colonies per plate)									
		Base-pair substitution type						Frameshift type			
		TA100		TA1535		WP2uvrA-pKM101		TA98		TA1537	
-	0	99 64 89	(84) 18.0#	24 25 25	(25) 0.6	126 114 105	(115) 10.5	18 19 17	(18) 1.0	18 18 22	(19) 2.3
-	0.5	94 80 87	(87) 7.0	24 25 29	(26) 2.6	99 94 114	(102) 10.4	14 15 19	(16) 2.6	11 15 17	(14) 3.1
-	1.5	87 93 85	(88) 4.2	20 22 24	(22) 2.0	89 91 110	(97) 11.6	18 20 23	(20) 2.5	9 13 15	(12) 3.1
-	5	90 85 88	(88) 2.5	25 26 19	(23) 3.8	116 92 104	(104) 12.0	15 26 21	(21) 5.5	18 8 15	(14) 5.1
-	15	85 89 86	(87) 2.1	20 27 22	(23) 3.6	110 103 118	(110) 7.5	26 27 18	(24) 4.9	7 9 22	(13) 8.1
-	50	90 89 87	(89) 1.5	22 23 27	(24) 2.6	129 115 99	(114) 15.0	29 24 16	(23) 6.6	18 10 11	(13) 4.4
-	150	40 S 47 S 37 S	(41) 5.1	10 V 11 V 19 V	(13) 4.9	87 S 85 S 73 S	(82) 7.6	11 V 7 V 4 V	(7) 3.5	4 V 13 V 4 V	(7) 5.2
Positive controls	Name	ENNG		ENNG		ENNG		4NQO		9AA	
	Concentration (µg/plate)	3		5		0.5		0.2		80	
S9-Mix -	No. colonies per plate	263 304 558	(375) 159.8	185 204 198	(196) 9.7	690 640 590	(640) 50.0	109 216 175	(167) 54.0	565 638 693	(632) 64.2

Table 5 Test Results: Experiment 2 – With Metabolic Activation

Test Period		From: 06 September 2007						To: 09 September 2007			
With or without S9-Mix	Test substance concentration (µg/plate)	Number of revertants (mean number of colonies per plate)									
		Base-pair substitution type						Frameshift type			
		TA100		TA1535		WP2uvrA-pKM101		TA98		TA1537	
+	0	66 67 65	(66) 1.0#	18 9 11	(13) 4.7	162 155 164	(160) 4.7	23 31 31	(28) 4.6	14 13 15	(14) 1.0
+	1.5	66 67 66	(66) 0.6	9 16 16	(14) 4.0	140 142 165	(149) 13.9	21 23 17	(20) 3.1	18 17 10	(15) 4.4
+	5	64 63 60	(62) 2.1	11 15 15	(14) 2.3	154 180 125	(153) 27.5	23 23 24	(23) 0.6	18 15 7	(13) 5.7
+	15	65 77 68	(70) 6.2	19 22 17	(19) 2.5	147 156 150	(151) 4.6	24 24 16	(21) 4.6	11 18 13	(14) 3.6
+	50	73 66 66	(68) 4.0	13 22 16	(17) 4.6	113 107 138	(119) 16.4	24 26 24	(25) 1.2	16 18 18	(17) 1.2
+	150	66 68 67	(67) 1.0	10 18 16	(15) 4.2	144 128 160	(144) 16.0	19 26 30	(25) 5.6	8 4 20	(11) 8.3
+	500	22 S 19 S 39 S	(27) 10.8	6 V 6 V 5 V	(6) 0.6	58 V 82 V 95 V	(78) 18.8	17 V 11 V 11 V	(13) 3.5	10 S 5 S 4 S	(6) 3.2
Positive controls	Name	2AA		2AA		2AA		BP		2AA	
	Concentration (µg/plate)	1		2		2		5		2	
S9-Mix +	No. colonies per plate	372 511 481	(455) 73.1	241 185 187	(204) 31.8	589 669 676	(645) 48.3	709 587 640	(645) 61.2	163 109 134	(135) 27.0

Study title: [redacted](b) (4)**: Reverse Mutation Assay "Ames Test" Using Salmonella typhimurium and Escherichia coli**

Key findings: Under the conditions of this study, [redacted](b) (4) impurity, is considered a mutagen.

Study no.: ED2008/000070/00 or 0102/0567

Volume and page #: Electronic submission
Conducting laboratory and location: [redacted](b) (4)
Date of study initiation:
GLP compliance: OECD
QA reports: yes
Drug, lot #, and % purity: [redacted](b) (4) Batch 15, purity not specified. The test article is also known as [redacted](b) (4) impurity.

Methods

Strains/species/cell line: *E. coli* TA98, TA100, TA1535 and TA1537 and *S. typhimurium* WP2*uvr*A

Doses used in definitive study: First assay: 50, 150, 500, 1500 and 5000 µg/plate
Second assay: 150, 500, 1500, 3000 and 5000 µg/plate

Basis of dose selection: Dose-range finding assay at 0, 0.15, 0.5, 1.5, 5, 15, 50, 150, 500, 1500 and 5000 µg/plate using TA 100 and WP2*uvr*A strains.

Negative controls: DMSO

Positive controls: N-ethyl-N'-nitro-N-nitrosoguanidine, 9-aminoacridine, 4-nitroquinoline-1-oxide, 2-aminoanthracene, benzo[a]pyrene

Incubation and sampling times: Standardized methodology with commercial S9 from male Sprague-Dawley rats was used.

Results

Study validity: The positive and negative controls performed as expected so this study is considered adequate for regulatory purposes.

Study outcome: No cytotoxicity was observed at any concentration tested and no precipitates were noted. A significant and reproducible increase in revertants was found in TA 100 with and without S9, TA 98 with S9 and TA 1535 with S9 (expt. 1 only) with concentrations of 1500- 5000 µg/plate.

Sponsor tables:

Table 2 Test Results: Experiment 1 – Without Metabolic Activation

Test Period		From: 09 August 2007						To: 12 August 2007			
With or without S9-Mix	Test substance concentration (µg/plate)	Number of revertants (mean number of colonies per plate)									
		Base-pair substitution type						Frameshift type			
		TA100		TA1535		WP2uvrA-pKM101		TA98		TA1537	
-	0	84 86 95	(88) 5.9#	24 16 19	(20) 4.0	95 96 102	(98) 3.8	18 13 22	(18) 4.5	5 8 9	(7) 2.1
-	50	102 122 88	(104) 17.1	17 24 9	(17) 7.5	101 84 87	(91) 9.1	16 15 17	(16) 1.0	14 4 4	(7) 5.8
-	150	92 104 120	(105) 14.0	19 8 18	(15) 6.1	67 75 73	(72) 4.2	20 14 14	(16) 3.5	4 2 3	(3) 1.0
-	500	138 139 132	(136) 3.8	23 24 19	(22) 2.6	81 81 89	(84) 4.6	17 8 21	(15) 6.7	9 7 5	(7) 2.0
-	1500	194 245 252	(230) 31.7	27 25 35	(29) 5.3	75 73 66	(71) 4.7	22 7 15	(15) 7.5	8 4 5	(6) 2.1
-	5000	240 147 141	(176) 55.5	24 27 34	(28) 5.1	72 62 86	(73) 12.1	27 22 26	(25) 2.6	6 4 3	(4) 1.5
Positive controls S9-Mix -	Name	ENNG		ENNG		ENNG		4NQO		9AA	
	Concentration (µg/plate)	3		5		0.5		0.2		80	
	No. colonies per plate	457 449 445	(450) 6.1	272 259 274	(268) 8.1	388 677 563	(543) 145.6	93 107 115	(105) 11.1	976 812 670	(819) 153.1

Table 3 Test Results: Experiment 1 – With Metabolic Activation

Test Period		From: 09 August 2007						To: 12 August 2007					
With or without S9-Mix	Test substance concentration (µg/plate)	Number of revertants (mean number of colonies per plate)											
		Base-pair substitution type						Frameshift type					
		TA100		TA1535		WP2uvrA-pKM101		TA98		TA1537			
+	0	95 95 64	(85) 17.9#	8 5 7	(7) 1.5	103 71 76	(83) 17.2	21 21 16	(19) 2.9	7 8 4	(6) 2.1		
+	50	98 103 80	(94) 12.1	7 7 6	(7) 0.6	94 104 92	(97) 6.4	19 15 32	(22) 8.9	5 10 8	(8) 2.5		
+	150	79 122 115	(105) 23.1	6 9 7	(7) 1.5	85 103 110	(99) 12.9	9 18 24	(17) 7.5	13 5 3	(7) 5.3		
+	500	133 148 178	(153) 22.9	8 14 9	(10) 3.2	118 109 94	(107) 12.1	20 19 23	(21) 2.1	7 6 8	(7) 1.0		
+	1500	232 232 158	(207) 42.7	16 16 13	(15) 1.7	109 96 98	(101) 7.0	31 19 31	(27) 6.9	7 4 4	(5) 1.7		
+	5000	487 401 422	(437) 44.8	20 23 28	(24) 4.0	110 94 80	(95) 15.0	45 33 46	(41) 7.2	7 2 5	(5) 2.5		
Positive controls S9-Mix +	Name	2AA		2AA		2AA		BP		2AA			
	Concentration (µg/plate)	1		2		2		5		2			
	No. colonies per plate	1159 1102 1069	(1110) 45.5	190 196 195	(194) 3.2	502 643 853	(666) 176.6	278 278 337	(298) 34.1	387 329 236	(317) 76.2		

Table 4 Test Results: Experiment 2 – Without Metabolic Activation

Test Period		From: 15 August 2007						To: 18 August 2007			
With or without S9-Mix	Test substance concentration (µg/plate)	Number of revertants (mean number of colonies per plate)									
		Base-pair substitution type						Frameshift type			
		TA100		TA1535		WP2uvrA-pKM101		TA98		TA1537	
-	0	78 68 63	(70) 7.6#	28 27 32	(29) 2.6	114 121 119	(118) 3.6	26 22 8	(19) 9.5	13 14 19	(15) 3.2
-	150	66 68 108	(81) 23.7	29 20 30	(26) 5.5	108 121 114	(114) 6.5	14 10 22	(15) 6.1	14 10 20	(15) 5.0
-	500	85 66 98	(83) 16.1	27 24 24	(25) 1.7	121 121 117	(120) 2.3	14 14 10	(13) 2.3	11 20 21	(17) 5.5
-	1500	121 98 107	(109) 11.6	38 30 39	(36) 4.9	117 104 107	(109) 6.8	22 19 17	(19) 2.5	14 22 25	(20) 5.7
-	3000	191 182 159	(177) 16.5	36 36 36	(36) 0.0	105 119 137	(120) 16.0	27 22 17	(22) 5.0	22 22 14	(19) 4.6
-	5000	337 274 305	(305) 31.5	37 32 45	(38) 6.6	126 97 125	(116) 16.5	30 34 26	(30) 4.0	20 10 8	(13) 6.4
Positive controls S9-Mix	Name	ENNG		ENNG		ENNG		4NQO		9AA	
-	Concentration (µg/plate)	3		5		0.5		0.2		80	
	No. colonies per plate	450 450 433	(444) 9.8	181 204 155	(180) 24.5	485 836 840	(720) 203.8	73 173 210	(152) 70.9	431 355 611	(466) 131.5

Table 5 Test Results: Experiment 2– With Metabolic Activation

Test Period		From: 15 August 2007						To: 18 August 2007			
With or without S9-Mix	Test substance concentration (µg/plate)	Number of revertants (mean number of colonies per plate)									
		Base-pair substitution type						Frameshift type			
		TA100		TA1535		WP2uvrA-pKM101		TA98		TA1537	
+	0	69 65 61	(65) 4.0#	23 13 10	(15) 6.8	208 232 272	(237) 32.3	18 23 24	(22) 3.2	11 22 13	(15) 5.9
+	150	61 61 62	(61) 0.6	13 8 11	(11) 2.5	206 195 141	(181) 34.8	24 21 24	(23) 1.7	14 18 26	(19) 6.1
+	500	69 79 79	(76) 5.8	10 11 14	(12) 2.1	201 127 136	(155) 40.4	24 29 20	(24) 4.5	16 15 17	(16) 1.0
+	1500	134 113 103	(117) 15.8	17 18 11	(15) 3.8	113 92 197	(134) 55.6	18 24 21	(21) 3.0	8 19 18	(15) 6.1
+	3000	204 175 205	(195) 17.0	26 30 30	(29) 2.3	112 138 111	(120) 15.3	26 39 39	(35) 7.5	21 21 25	(22) 2.3
+	5000	426 287 374	(362) 70.2	32 36 28	(32) 4.0	127 166 201	(165) 37.0	55 48 46	(50) 4.7	13 24 15	(17) 5.9
Positive controls S9-Mix +	Name	2AA		2AA		2AA		BP		2AA	
	Concentration (µg/plate)	1		2		2		5		2	
	No. colonies per plate	483 632 653	(589) 92.7	203 191 280	(225) 48.3	736 745 734	(738) 5.9	286 294 336	(305) 26.9	297 266 283	(282) 15.5

Study title: [redacted] **Reverse Mutation Assay "Ames Test" Using Salmonella typhimurium and Escherichia coli**

Key findings: A significant and reproducible increase in revertants was appreciated with TA100, TA1535 and WP2*uvr*A with and without metabolic activation. No significant increases were found with TA98 or TA1537.

Under the conditions of this study, [redacted] impurity, is considered a mutagen.

Study no.: CD2008/00969/00 or 0102/0618

Volume and page #: Electronic submission
Conducting laboratory and location: [redacted]
Date of study initiation: 2/26/08
GLP compliance: Yes
QA reports: yes
Drug, lot #, and % purity: [redacted], Batch 209401-194 at 99.6% purity; test article is also known at [redacted] impurity.

Methods

Strains/species/cell line: *E. coli* TA98, TA100, TA1535 and TA1537 and *S. typhimurium* WP2*uvr*A

Doses used in definitive study: First assay: *Salmonella strain*: 1.5, 5, 15, 50, 150, 500 and 1500 µg/plate; *E. coli*: 5, 15, 150, 500, 1500 and 5000 µg/plate
Second assay: Same as in the first assay

Basis of dose selection: Dose-range finding assay at 0, 0.15, 0.5, 1.5, 5, 15, 50, 150, 500, 1500 and 5000 µg/plate using TA 100 and WP2*uvr*A strains. Decreased bacterial lawns were found at >500 µg/plate.

Negative controls: DMSO

Positive controls: N-ethyl-N'-nitro-N-nitrosoguanidine, 9-aminoacridine, 4-nitroquinoline-1-oxide, 2-aminoanthracene, benzo[a]pyrene

Incubation and sampling times: Standardized methodology

Results

Study validity: The positive and negative controls performed adequately so this study is considered valid for regulatory purposes.

Study outcome: A significant decrease in the bacterial lawn was found in all strains at ≥500 µg/plate but the variability was high. No precipitates were seen at any concentration.

A significant and reproducible increase in revertants was appreciated with TA100, TA1535 and WP2*uvr*A with and without metabolic activation. No significant increases were found with TA98 or TA1537.

Under the conditions of this study, (b) (4) is considered a mutagen.

Sponsor tables:

Table 2 Test Results: Experiment 1 – Without Metabolic Activation

Test Period		From: 01 March 2008						To: 04 March 2008			
With or without S9-Mix	Test substance concentration (µg/plate)	Number of revertants (mean number of colonies per plate)									
		Base-pair substitution type						Frameshift type			
		TA100		TA1535		WP2uvrA-pKM101		TA98		TA1537	
-	0	95 100 90	(95) 5.0#	34 22 32	(29) 6.4	203 205 206	(205) 1.5	20 15 15	(17) 2.9	8 5 8	(7) 1.7
-	1.5	76 101 73	(83) 15.4	34 27 31	(31) 3.5	NT		15 23 12	(17) 5.7	7 13 13	(11) 3.5
-	5	76 100 115	(97) 19.7	34 30 36	(33) 3.1	201 205 235	(214) 18.6	27 25 15	(22) 6.4	11 10 10	(10) 0.6
-	15	90 109 93	(97) 10.2	36 32 32	(33) 2.3	216 249 229	(231) 16.6	21 20 16	(19) 2.6	7 7 9	(8) 1.2
-	50	98 99 120	(106) 12.4	106 102 114	(107) 6.1	271 272 285	(276) 7.8	24 26 16	(22) 5.3	11 8 4	(8) 3.5
-	150	126 103 102	(110) 13.6	102 114 115	(110) 7.2	334 377 386	(366) 27.8	23 23 13	(20) 5.8	10 10 11	(10) 0.6
-	500	63 S 66 S 62 S	(64) 2.1	40 S 37 S 41 S	(39) 2.1	208 228 211	(216) 10.8	18 S 14 S 10 S	(14) 4.0	5 S 11 S 9 S	(8) 3.1
-	1500	0 V 0 V 0 V	(0) 0.0	0 V 0 V 0 V	(0) 0.0	102 S 93 S 98 S	(98) 4.5	0 V 0 V 0 V	(0) 0.0	0 V 0 V 0 V	(0) 0.0
-	5000	NT		NT		0 V 0 V 0 V	(0) 0.0	NT		NT	
Positive controls S9-Mix -	Name	ENNG		ENNG		ENNG		4NQO		9AA	
	Concentration (µg/plate)	3		5		0.5		0.2		80	
	No. colonies per plate	592 588 572	(584) 10.6	363 398 360	(374) 21.1	767 840 894	(834) 63.7	121 139 110	(123) 14.6	477 525 565	(522) 44.1

BP Benzo(a)pyrene
2AA 2-Aminoanthracene
NT Not tested at this dose level
S Sparse bacterial background lawn
V Very weak bacterial background lawn
Standard deviation

Table 3 Test Results: Experiment 1 – With Metabolic Activation

Test Period		From: 01 March 2008						To: 04 March 2008			
With or without S9-Mix	Test substance concentration (µg/plate)	Number of revertants (mean number of colonies per plate)									
		Base-pair substitution type						Frameshift type			
		TA100		TA1535		WP2uvrA-pKM101		TA98		TA1537	
+	0	100 93 80	(91) 10.1#	24 30 32	(29) 4.2	209 235 242	(229) 17.4	19 29 31	(26) 6.4	12 14 13	(13) 1.0
+	1.5	100 86 101	(96) 8.4	27 33 36	(32) 4.6	NT		32 27 26	(28) 3.2	12 13 10	(12) 1.5
+	5	89 109 98	(99) 10.0	33 25 44	(34) 9.5	239 245 236	(240) 4.6	22 24 26	(24) 2.0	20 13 5	(13) 7.5
+	15	117 115 119	(117) 2.0	45 59 60	(55) 8.4	266 294 284	(281) 14.2	20 15 19	(18) 2.6	13 8 14	(12) 3.2
+	50	158 189 208	(185) 25.2	115 92 124	(110) 16.5	350 330 311	(330) 19.5	26 30 32	(29) 3.1	13 8 18	(13) 5.0
+	150	230 232 297	(253) 38.1	199 175 164	(179) 17.9	487 498 446	(477) 27.4	23 33 25	(27) 5.3	16 7 16	(13) 5.2
+	500	89 S 73 S 93 S	(85) 10.6	163 S 142 S 153 S	(153) 10.5	439 476 459	(458) 18.5	20 S 16 S 21 S	(19) 2.6	12 8 14	(11) 3.1
+	1500	0 V 0 V 0 V	(0) 0.0	0 V 0 V 0 V	(0) 0.0	128 S 126 S 113 S	(122) 8.1	0 V 0 V 0 V	(0) 0.0	5 S 11 S 5 S	(7) 3.5
+	5000	NT		NT		0 V 0 V 0 V	(0) 0.0	NT		NT	
Positive controls S9-Mix	Name	2AA		2AA		2AA		BP		2AA	
+	Concentration (µg/plate)	1		2		10		5		2	
	No. colonies per plate	908 1094 1172	(1058) 135.6	210 206 186	(201) 12.9	776 756 863	(798) 56.9	189 139 197	(175) 31.4	188 225 220	(211) 20.1

Second assay:

Table 4 Test Results: Experiment 2 – Without Metabolic Activation

Test Period		From: 09 March 2008			To: 12 March 2008							
With or without S9-Mix	Test substance concentration (µg/plate)	Number of revertants (mean number of colonies per plate)										
		Base-pair substitution type							Frameshift type			
		TA100			TA1535			WP2uvrA-pKM101			TA98			TA1537		
-	0	110 109 111	(110) 1.0#		28 29 26	(28) 1.5		96 129 131	(119) 19.7		17 22 22	(20) 2.9		11 13 6	(10) 3.6	
-	1.5	106 120 101	(109) 9.8		27 33 25	(28) 4.2		NT			14 17 15	(15) 1.5		9 5 3	(6) 3.1	
-	5	95 120 141	(119) 23.0		32 44 33	(36) 6.7		132 109 102	(114) 15.7		10 14 12	(12) 2.0		12 3 6	(7) 4.6	
-	15	149 228 227	(201) 45.3		46 51 53	(50) 3.6		138 145 105	(129) 21.4		14 20 19	(18) 3.2		13 14 3	(10) 6.1	
-	50	141 237 219	(199) 51.0		97 107 107	(104) 5.8		167 173 165	(168) 4.2		19 17 20	(19) 1.5		6 10 14	(10) 4.0	
-	150	231 247 216	(231) 15.5		153 148 135	(145) 9.3		265 247 258	(257) 9.1		16 17 22	(18) 3.2		10 13 7	(10) 3.0	
-	500	65 S 62 S 60 S	(62) 2.5		34 S 18 S 19 S	(24) 9.0		111 116 130	(119) 9.8		7 S 6 S 7 S	(7) 0.6		2 S 5 S 4 S	(4) 1.5	
-	1500	0 V 0 V 0 V	(0) 0.0		0 V 0 V 0 V	(0) 0.0		56 S 37 S 32 S	(42) 12.7		0 V 0 V 0 V	(0) 0.0		0 V 0 V 0 V	(0) 0.0	
-	5000	NT			NT			0 V 0 V 0 V	(0) 0.0		NT			NT		
Positive controls	Name	ENNG			ENNG			ENNG			4NQO			9AA		
	Concentration (µg/plate)	3			5			0.5			0.2			80		
S9-Mix	No. colonies per plate	309 504 638	(484) 165.4		209 253 233	(232) 22.0		686 680 660	(675) 13.6		126 130 111	(122) 10.0		865 607 990	(821) 195.3	
-																

Table 5 Test Results: Experiment 2 – With Metabolic Activation

Test Period		From: 09 March 2008						To: 12 March 2008					
With or without S9-Mix	Test substance concentration (µg/plate)	Number of revertants (mean number of colonies per plate)											
		Base-pair substitution type								Frameshift type			
		TA100		TA1535		WP2uvrA-pKM101		TA98		TA1537			
+	0	86 101 125	(104) 19.7#	15 11 12	(13) 2.1	194 177 211	(194) 17.0	26 18 14	(19) 6.1	15 17 11	(14) 3.1		
+	1.5	114 99 90	(101) 12.1	15 10 13	(13) 2.5	NT		19 28 22	(23) 4.6	15 13 6	(11) 4.7		
+	5	106 111 110	(109) 2.6	28 26 15	(23) 7.0	234 239 244	(239) 5.0	17 21 26	(21) 4.5	14 10 10	(11) 2.3		
+	15	124 137 177	(146) 27.6	42 46 37	(42) 4.5	226 269 247	(247) 21.5	18 23 34	(25) 8.2	6 12 10	(9) 3.1		
+	50	259 260 274	(264) 8.4	115 123 105	(114) 9.0	347 368 412	(376) 33.2	30 27 26	(28) 2.1	9 7 7	(8) 1.2		
+	150	303 366 433	(367) 65.0	162 231 189	(194) 34.8	495 497 558	(517) 35.8	19 27 27	(24) 4.6	4 10 10	(8) 3.5		
+	500	97 S 84 S 57 S	(79) 20.4	31 S 26 S 22 S	(26) 4.5	341 274 271	(295) 39.6	17 S 22 S 12 S	(17) 5.0	3 7 6	(5) 2.1		
+	1500	0 V 0 V 0 V	(0) 0.0	0 V 0 V 0 V	(0) 0.0	62 S 63 S 51 S	(59) 6.7	0 V 0 V 0 V	(0) 0.0	6 S 3 S 7 S	(5) 2.1		
+	5000	NT		NT		0 V 0 V 0 V	(0) 0.0	NT		NT			
Positive controls	Name	2AA		2AA		2AA		BP		2AA			
	Concentration (µg/plate) No. colonies per plate	1		2		2		5		2			
S9-Mix +		1564 1890 1754	(1736) 163.7	268 195 205	(223) 39.6	1866 2161 1463	(1830) 350.4	187 167 221	(192) 27.3	192 297 220	(236) 54.4		

2.6.6.5 Carcinogenicity

Study title: **104-Week Oral Carcinogenicity Study of XP13512 in Mice**

Key study findings: In mice treated with 0, 500, 2000 or 5000 mg/kg/d by oral gavage, XP13512 treatment caused decreased survival in the mid and high dose males and increased body weights in the high dose animals. No other significant findings were appreciated in treated animals except for a modest exacerbation of age-related axonal/myelin degeneration of the sciatic nerve in the females at 2000 mg/kg/d and both sexes at 5000 mg/kg/d.

Adequacy of the carcinogenicity study and appropriateness of the test model:
This appears to have been a well conducted study in an appropriate animal model.
Evaluation of tumor findings: There were no increases in any tumor types elicited by treatment with XP13512.

Study no.: RD2008/00346/00XP050 or 1032-047
Volume and page #: Electronic submission
Conducting laboratory and location: (b) (4)
Date of study initiation: 6/15/05
GLP compliance: Yes
QA report: yes
Drug, lot #, and % purity: Batches 14, 30, 31, 29, 43 and 64 at >98% purity
CAC concurrence: Yes; minutes appended

Methods

Doses: 0, 500, 2000 or 5000 mg/kg/d
Basis of dose selection: MFD
Species/strain: B6C3F$_1$/Crl mice
Number/sex/group (main study): 60
Route, formulation, volume: Oral gavage in 0.1% v/v Tween®80 and 0.5% w/v methylcellulose at 20 mL/kg
Frequency of dosing: Daily
Satellite groups used for toxicokinetics or special groups: N/A
Age: Approximately 8 weeks of age at study initiation
Animal housing: Individually during the dosing period
Restriction paradigm for dietary restriction studies: N/A
Drug stability/homogeneity: Dosing solutions were stable and homogeneous
Dual controls employed: No
Interim sacrifices: None
Deviations from original study protocol: None of significance

Observation times
Mortality: Twice daily for the first year and thrice daily thereafter
Clinical signs: Twice daily

Body weights: Weekly for the first 14 weeks, then every 2 weeks until Week 28, then every 4 weeks until study termination
Food consumption: Weekly for the first 14 weeks, every 2 weeks for 14 weeks, then every 4 weeks
Hematology: All premature decedents sacrificed in extremis and at study termination
Histopathology: All groups were examined.
Peer review: yes by an in-house pathologist for all tumors and hyperplastic lesions and 10% of the control and high dose animals. An adequate battery of tissues was examined.
Toxicokinetics: Not evaluated

Results
Mortality: Survival was adversely affected in the 2000 and 5000 mg/kg/d males but enough animals survived to consider the study valid (Week 78: 92%, 83%, 83% and 83% for the respective male groups). The decreased survival was more evident towards the end of the study. Although decreased survival is often noted after Week 78 in mouse carcinogenicity studies, it appears that treatment with XP13512 statistically significantly increased mortality in mid and high dose males.

Sponsor table:

Table 1 **Summary of Survival Estimates - MALE**

Dosage Level	Study Interval (Week)	Deaths	Censored	Effective Sample Size	Cumulative Survival	Survival Standard Error
0 mg/kg/day (Vehicle Control)[z]						
	1-13	1	0	60.0	1.0000	0.0000
	14-26	3	0	59.0	0.9833	0.0165
	27-39	1	0	56.0	0.9333	0.0322
	40-52	0	0	55.0	0.9167	0.0357
	53-65	0	0	55.0	0.9167	0.0357
	66-78	0	0	55.0	0.9167	0.0357
	79-91	1	0	55.0	0.9167	0.0357
	92-104	4	0	54.0	0.9000	0.0387
	105	1	49[&]	25.5	0.8333	0.0481
500 mg/kg/day						
	1-13	1	0	60.0	1.0000	0.0000
	14-26	3	0	59.0	0.9833	0.0165
	27-39	0	0	56.0	0.9333	0.0322
	40-52	1	0	56.0	0.9333	0.0322
	53-65	3	0	55.0	0.9167	0.0357
	66-78	2	0	52.0	0.8667	0.0439
	79-91	3	0	50.0	0.8333	0.0481
	92-104	5	0	47.0	0.7833	0.0532
	105	0	42[&]	21.0	0.7000	0.0592
2000 mg/kg/day[a]						
	1-13	0	0	60.0	1.0000	0.0000
	14-26	0	0	60.0	1.0000	0.0000
	27-39	1	0	60.0	1.0000	0.0000
	40-52	3	0	59.0	0.9833	0.0165
	53-65	4	0	56.0	0.9333	0.0322
	66-78	2	0	52.0	0.8667	0.0439
	79-91	5	0	50.0	0.8333	0.0481
	92-104	7	0	45.0	0.7500	0.0559
	105	1	37[&]	19.5	0.6333	0.0622
5000 mg/kg/day[b]						
	1-13	1	0	60.0	1.0000	0.0000
	14-26	1	0	59.0	0.9833	0.0165
	27-39	3	0	58.0	0.9667	0.0232
	40-52	0	0	55.0	0.9167	0.0357
	53-65	3	0	55.0	0.9167	0.0357
	66-78	2	0	52.0	0.8667	0.0439
	79-91	11	0	50.0	0.8333	0.0481
	92-104	9	0	39.0	0.6500	0.0616
	105	0	30[&]	15.0	0.5000	0.0645

[&]This is the necropsy count
[z]Statistically significant for overall test at p<0.05
[a]Significantly different from control; (p<0.05)
[b]Significantly different from control; (p<0.01)

Table 1 Summary of Survival Estimates - FEMALE

Dosage Level	Study Interval (Week)	Deaths	Censored	Effective Sample Size	Cumulative Survival	Survival Standard Error
0 mg/kg/day (Vehicle Control)						
	1-13	2	0	60.0	1.0000	0.0000
	14-26	0	0	58.0	0.9667	0.0232
	27-39	2	0	58.0	0.9667	0.0232
	40-52	1	0	56.0	0.9333	0.0322
	53-65	0	0	55.0	0.9167	0.0357
	66-78	3	0	55.0	0.9167	0.0357
	79-91	3	0	52.0	0.8667	0.0439
	92-104	12	0	49.0	0.8167	0.0500
	105	1	36[&]	19.0	0.6167	0.0628
500 mg/kg/day						
	1-13	1	0	60.0	1.0000	0.0000
	14-26	0	0	59.0	0.9833	0.0165
	27-39	1	0	59.0	0.9833	0.0165
	40-52	0	0	58.0	0.9667	0.0232
	53-65	0	0	58.0	0.9667	0.0232
	66-78	2	0	58.0	0.9667	0.0232
	79-91	4	0	56.0	0.9333	0.0322
	92-104	12	0	52.0	0.8667	0.0439
	105	1	39[&]	20.5	0.6667	0.0609
2000 mg/kg/day						
	1-13	0	0	60.0	1.0000	0.0000
	14-26	1	0	60.0	1.0000	0.0000
	27-39	2	0	59.0	0.9833	0.0165
	40-52	2	0	57.0	0.9500	0.0281
	53-65	1	0	55.0	0.9167	0.0357
	66-78	2	0	54.0	0.9000	0.0387
	79-91	3	0	52.0	0.8667	0.0439
	92-104	8	0	49.0	0.8167	0.0500
	105	3	38[&]	22.0	0.6833	0.0601
5000 mg/kg/day						
	1-13	0	0	60.0	1.0000	0.0000
	14-26	1	0	60.0	1.0000	0.0000
	27-39	4	0	59.0	0.9833	0.0165
	40-52	0	0	55.0	0.9167	0.0357
	53-65	1	0	55.0	0.9167	0.0357
	66-78	1	0	54.0	0.9000	0.0387
	79-91	5	0	53.0	0.8833	0.0414
	92-104	6	0	48.0	0.8000	0.0516
	105	0	42[&]	21.0	0.7000	0.0592

[&]This is the necropsy count

Clinical signs: No adverse treatment-related clinical signs were observed.

Body weights: Body weights were increased in the high dose animals of both sexes.

Food consumption: No intergroup differences were found.

Summary of Neoplastic Lesions and Table of Tumor-Bearing Animals - MALE

Table 10 Terminal

Tissue Diagnosis	0 mg/kg/day (Vehicle Control) No. with Tumor	Animal No.	Fate/ Day	500 mg/kg/day No. with Tumor	Animal No.	Fate/ Day	2000 mg/kg/day No. with Tumor	Animal No.	Fate/ Day	5000 mg No. with Tumor	Ani N
lung	(60)			(60)			(60)			(60)	
carcinoma, hepatocellular, malignant, secondary	1	1039	D 733	1	1084	S 730	1	1138	D 552	1	12
fibrosarcoma, malignant, secondary	0			0			2	1166 1174	E 615 D 559	0	
lymph node, axillary	(1)			(0)			(2)			(0)	
fibrosarcoma, malignant, secondary	0			0			1	1166	E 615	0	
lymph node, hepatic	(0)			(1)			(2)			(4)	
lymph node, inguinal	(2)			(1)			(1)			(0)	
lymph node, mandibular	(56)			(52)			(53)			(56)	
fibrosarcoma, malignant, secondary	0			0			1	1166	E 615	0	
lymph node, mediastinal	(0)			(1)			(0)			(1)	
lymph node, mesenteric	(58)			(56)			(53)			(59)	
lymph node, renal	(2)			(0)			(0)			(0)	

S - Scheduled Necropsy E - Euthanized *in extremis* D - Died on Study
No. - Number ( ) - Total number examined

There was an increased incidence of pancreatic acinar cell hyperplasia, adenomas and carcinomas in both sexes at 5000 mg/kg/d and in males at 2000 mg/kg/d. Thus XP13512 is considered a carcinogen in rats under the conditions of this study.

Adequacy of the carcinogenicity study and appropriateness of the test model: Wistar rats are commonly used for carcinogenicity testing. The study appears to have been appropriately conducted and the mid and high doses elicited toxicity as well as tumors.

Evaluation of tumor findings: An increased incidence of pancreatic acinar adenomas and adenocarcinomas were found at 5000 mg/kg/d in both sexes and a trend towards an increase was also noted in the 2000 mg/kg/d males. Although the 2000 mg/kg/d males had slightly increased severity of the hyperplasia, there was an increased incidence of adenomas and a carcinoma was found. The decreased survival and early termination in the 2000 and 5000 mg/kg/d males may be responsible for a lesser incidence of both non-neoplastic and neoplastic lesions.

Study no.: RD2008/00347/00 or XP051 or 1032-048
Volume and page #: Electronic submission
Conducting laboratory and location: (b) (4)
Date of study initiation: 6/21/05

Summary of Neoplastic Lesions and Table of Tumor-Bearing Animals - FEMALE

Table 10 Terminal

Tissue Diagnosis	0 mg/kg/day (Vehicle Control) No. with Tumor	Animal No.	Fate/ Day	500 mg/kg/day No. with Tumor	Animal No.	Fate/ Day	2000 mg/kg/day No. with Tumor	Animal No.	Fate/ Day	5000 mg/kg/day No. with Tumor	Animal No.	Fate/ Day
tail	(0)			(0)			(1)			(3)		
thymus gland	(58)			(57)			(58)			(56)		
thyroid gland	(58)			(60)			(60)			(60)		
adenoma, follicular cell, benign, primary	1	1289	S 730	2	1332 1365	D 727 S 734	0			1	1505	S 735
tongue	(60)			(60)			(60)			(60)		
trachea	(58)			(60)			(60)			(58)		
ureters	(60)			(59)			(57)			(57)		
carcinoma, squamous cell, malignant, secondary	0			1	1378	D 700	0			0		
schwannoma, malignant, secondary	0			0			1	1405	D 724	0		
urinary bladder	(60)			(60)			(60)			(60)		
sarcoma, undifferentiated, malignant, secondary	1	1307	E 692	0			0			0		

S - Scheduled Necropsy E - Euthanized *in extremis* D - Died on Study
No. - Number ( ) - Total number examined

Dual controls employed: No
Interim sacrifices: None
Deviations from original study protocol: Group 3 males were dosed through Week 97; group 4 males through Week 90

Observation times
Mortality: Twice/day for the first year, thrice/day thereafter
Clinical signs: As for mortality
Body weights: Weekly for 14 weeks, every 14 days until Week 28 and monthly thereafter
Food consumption: As for body weights
Hematology: From all premature decedents and all animals prior to sacrifice
Histopathology: A full tissue battery was examined for animals on study
Peer review: yes by an in-house pathologist for all neoplasms, target tissues

Results
Mortality: Survival was decreased in the mid and high dose animals of both sexes with males terminated early (Week 90 for high dose, Week 97 for mid dose).

Sponsor table:

Table 7 Summary of Probable Cause of Death - MALE				
Cause of Death	0 mg/kg/day (Vehicle Control)	500 mg/kg/day	2000 mg/kg/day	5000 mg/kg/day
Number of Animals	60	60	60	60
Summary of Animal Disposition				
accidental death	0	0	1	0
died after dosing	0	1	0	0
died prior to euthanasia	2	2	0	1
euthanized *in extremis*	11	13	11	8
found dead	26	19	33	36
terminal necropsy	21	25	15	15
Cause of Death				
abdominal mass	0	0	1	0
accessory sex gland tumor	0	0	1	0
accidental injury	0	0	1	0
brain tumor	0	0	1	1
chronic progressive nephropathy/uremia	12	9	20	26
dosing error	0	2	1	6
fibrosarcoma/fibroma	3	2	2	0
hemangiosarcoma/hemangioma	0	1	0	1
hemorrhage	2	0	1	0
histiocytic sarcoma	1	0	0	0
inflammation/septicemia	3	0	1	0
intestinal adenocarcinoma	0	0	0	1
kidney tumor	0	0	0	1
liposarcoma	1	0	0	0
liver tumor	0	0	0	1
lymphoid tumor	0	1	1	0
nose/oral tumor	1	1	3	0
pancreas tumor	0	0	1	0
pituitary tumor	4	3	4	1
polyarteritis	0	1	0	0
schwannoma	2	1	0	0

Table 7 Summary of Probable Cause of Death - FEMALE				
Cause of Death	0 mg/kg/day (Vehicle Control)	500 mg/kg/day	2000 mg/kg/day	5000 mg/kg/day
Number of Animals	60	60	60	60
Summary of Animal Disposition				
died prior to euthanasia	1	0	0	0
euthanized *in extremis*	8	15	22	13
found dead	6	7	16	25
terminal necropsy	45	38	22	22
Cause of Death				
adrenal gland tumor	0	0	0	1
brain tumor	0	1	1	1
carcinoma	0	0	1	0
chronic progressive nephropathy/uremia	0	0	1	4
dosing error	1	0	2	5
fibrosarcoma/fibroma	1	0	2	0
hemorrhage	1	0	0	0
inflammation/septicemia	0	1	0	0
kidney tumor	0	0	1	0
liposarcoma	0	1	0	0
liver inflammation/necrosis	0	0	1	0
lung inflammation/necrosis	0	1	0	1
lung tumor	1	0	0	0
lymphoid tumor	0	0	2	1
mammary tumor	4	3	10	7
mesothelioma	0	1	0	0
nose/oral tumor	0	0	1	0
odontodysplasia/periodontitis	1	0	0	0
ovarian cyst/hemorrhage	0	1	0	1
pituitary tumor	4	7	7	11
schwannoma	1	0	0	0
skin tumor	0	0	1	0
undetermined	1	4	2	4

Figure 1 Mean Survival Estimate – MALE

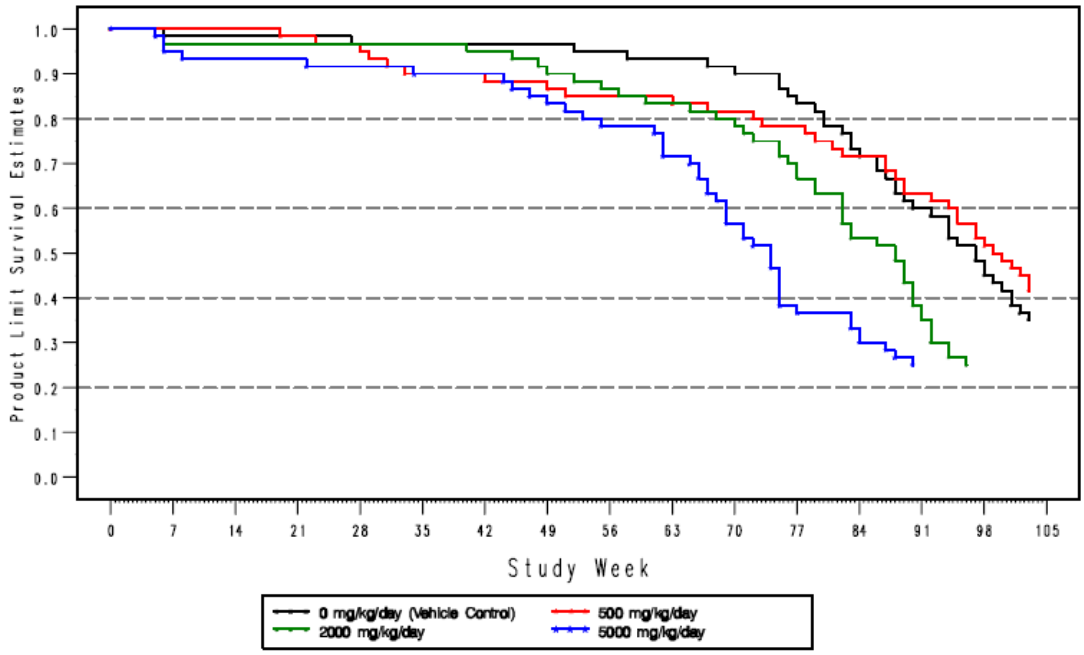
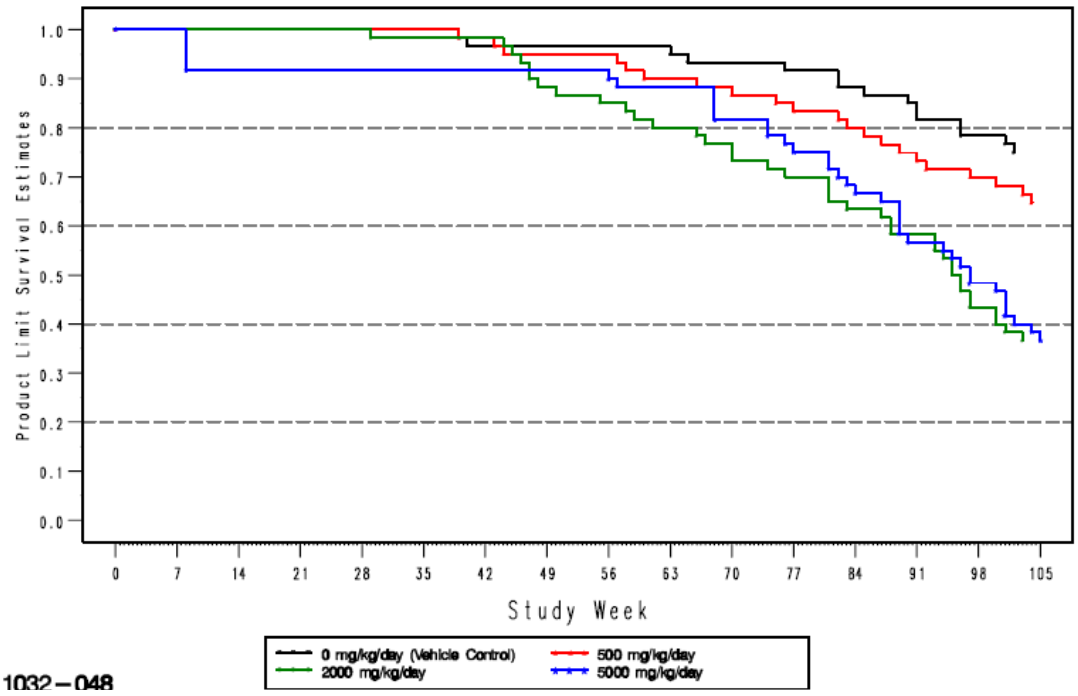


Figure 1A Mean Survival Estimate – FEMALE



1032-048

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Clinical signs: Increased salivation at the high dose was the only consistent, treatment-related adverse clinical sign reported. Rales and audible breathing were recorded for the mid and high dose groups (males: 5, 9, 12 and 27; females: 1, 4, 8, and 38 for the respective groups) and appear to be consistent with aspiration of the test article.

Histologic correlates of alveolar histiocytosis and/or chronic inflammation were found in the mid and high dose males but in all dose groups in females.

Sponsor table:

Selected Microscopic Findings: Lung
Terminal Necropsy
Male and Female

Dose Level (mg/kg/day)	0		500		2000		5000	
Sex	M	F	M	F	M	F	M	F
Number Examined	60	60	60	60	60	60	60	60
Lung								
Histiocytosis, alveolar	25	14	27	24	26	36	39	46
- minimal	19	8	18	15	18	18	13	17
- mild	4	5	8	7	8	14	19	20
- moderate	1	1	1	2	0	4	6	9
- severe	1	0	0	0	0	0	1	0
Inflammation, chronic	7	4	6	11	11	18	19	26
- minimal	6	4	2	7	8	6	5	15
- mild	1	0	4	4	3	12	14	9
- moderate	0	0	0	0	0	0	0	2

Clonic convulsions were noted in all dose groups (males: 7, 24, 4, 1 and females: 4, 12, 6 and 3 for the respective groups). As dose dependency was not seen and the mid and high dose groups had fewer than the low dose group, it is difficult to attribute the convulsions to XP13512 treatment. According to the sponsor, the incidences are within the historical control range for clonic convulsions at the laboratory.

Body weights: Body weights were increased (≤7%) in the mid dose males (occasionally statistically significant) and high dose animals (both sexes: consistently statistically significant for the first 52 weeks). This finding is not considered toxicologically significant in this study.

Figure 2 Mean Body Weight Values – MALE

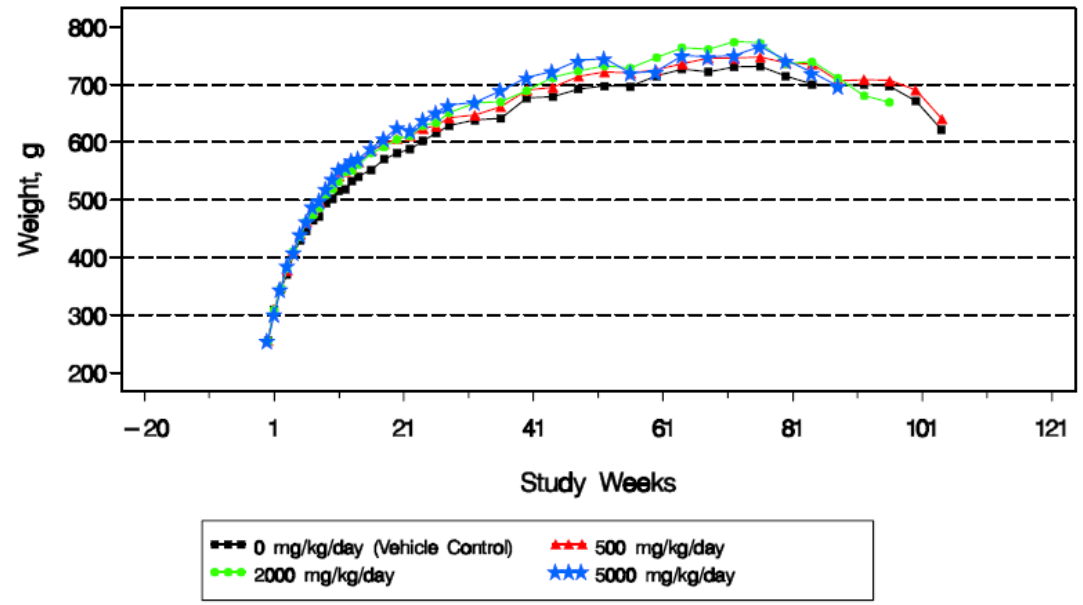
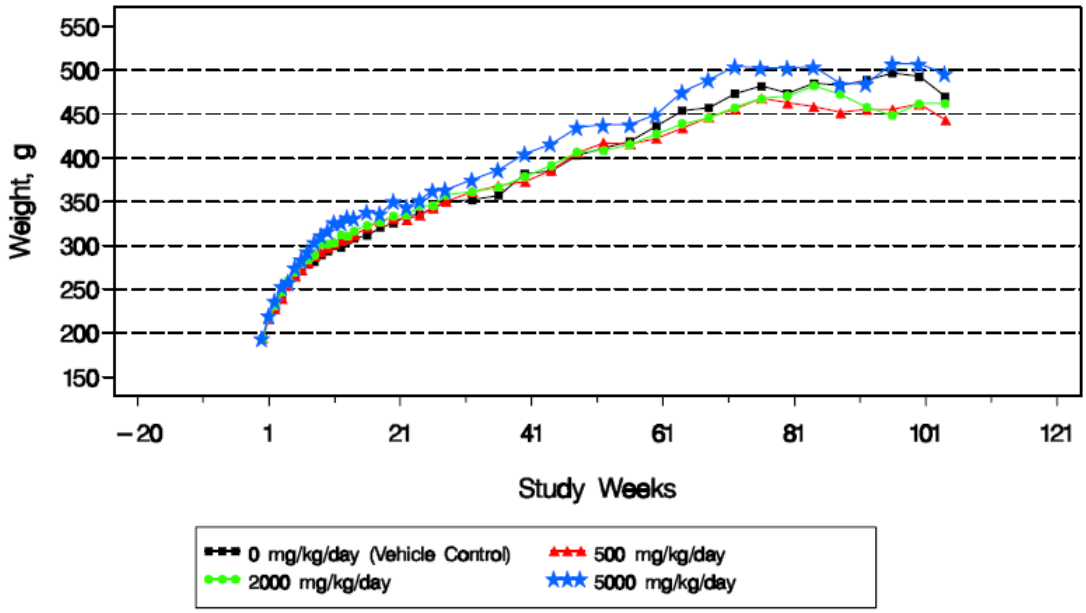


Figure 2A Mean Body Weight Values – FEMALE



Food consumption: Consumption was increased in mid dose males and both sexes at the high dose.

Hematology: No adverse effects of XP13512 were found.

Gross pathology: Enlarged kidneys were described in the mid dose males, especially the premature decedents (10/45 vs. 4/39). This finding correlated with an increased

incidence and severity of chronic progressive nephropathy and it was considered the cause of death in most of the premature decedents.

Histopathology:

Non-neoplastic: Pancreatic acinar cell hyperplasia increased in incidence and severity with increasing dose. This finding is considered associated with XP13512 treatment as increased neoplasms were found (see table below).

An exacerbation of age-related chronic progressive nephropathy was determined in the mid and high dose males, especially the premature decedents (see tables below).

Sponsor tables:

Incidence of Deaths Due to CPN/Uremia
Died on Study
Male and Female

Dose Level (mg/kg/day)	0		500		2000		5000	
Sex	M	F	M	F	M	F	M	F
Died on Study	**39**	**15**	**35**	**22**	**45**	**38**	**45**	**38**
Chronic Progressive Nephropathy/Uremia	12	0	9	0	20	1	26	4

Selected Microscopic Findings: Kidneys
Terminal Necropsy
Male and Female

Dose Level (mg/kg/day)	0		500		2000		5000	
Sex	M	F	M	F	M	F	M	F
Number Examined	**60**	**60**	**60**	**60**	**60**	**60**	**60**	**60**
Kidneys								
Nephropathy, chronic progressive	57	59	57	54	57	52	57	53
- minimal	4	37	4	39	5	30	4	25
- mild	12	13	21	10	10	17	9	11
- moderate	14	7	12	5	9	2	7	10
- severe	27	2	20	0	33	3	37	7

Centrilobular and/or midzonal hepatocyte vacuolation was seen in the mid dose males and high dose animals of both sexes, primarily in the premature decedents and both areas of distribution were often in the same animals. Although similar lesions were not reported for the controls, it seems reasonable to relate the usually minimal to mild vacuolar change to the poor condition of the animals and it is considered related to treatment.

Selected Microscopic Findings: Liver Terminal Necropsy Male and Female								
Dose Level (mg/kg/day)	**0**		**500**		**2000**		**5000**	
Sex	**M**	**F**	**M**	**F**	**M**	**F**	**M**	**F**
Number Examined	**60**	**60**	**60**	**60**	**60**	**60**	**60**	**60**
Liver								
Vacuolation, centrilobular	0	0	0	0	4	2	22	8
- minimal	0	0	0	0	0	1	3	4
- mild	0	0	0	0	4	1	14	4
- moderate	0	0	0	0	0	0	5	0
Vacuolation, midzonal	15	3	11	15	27	6	22	22
- minimal	8	2	6	15	20	5	10	21
- mild	6	1	1	0	7	0	9	1
- moderate	0	0	1	0	0	1	3	0
- severe	1	0	3	0	0	0	0	0

Neoplastic: Pancreatic acinar cell hyperplasia as well as adenomas/ adenocarcinomas were increased in the 5000 mg/kg/d animals with males more affected than females. The incidence of adenomas at the high dose exceeded the historical control range for this laboratory (0- 8.3%). The incidence of these lesions was also increased in the mid-dose group. No metastases were described in any of the affected animals. Statistical significance in males was approached with the Fisher's Exact Test (p=0.095 – not significant) and the Cochran-Armitage Trend Test (p= 0.02) for combined tumors at 5000 mg/kg/d; for females the Fisher's Exact Test (not significant) and the Cochran-Armitage Trend Test (p=0.07) for combined tumors.

Combined Pancreatic Lesions in Rats Treated with XP13512 for Up to 104 Weeks

	Males				**Females**			
Dose (mg/kg/d)	**0**	**500**	**2000**	**5000**	**0**	**500**	**2000**	**5000**
Hyperplasia, acinar; min-mild	11	8	11	17	1	0	3	10
Mod-severe	3	2	3	3	0	1	1	4
Acinar adenoma	2	4	4	8	0	0	0	3
Acinar carcinoma	0	0	1	1	0	0	0	1

Granular cell tumors of the uterus were determined for all dose groups when compared to controls (1, 3, 3, and 7 for the respective groups). Statistical significance was not reached. In the 5000 mg/kg/d females, an increased incidence of granular cell tumors of the vagina

was also appreciated. However all of these tumors were benign. In a recent publication with a comprehensive evaluation of the reproductive tracts from control females in 9 carcinogenicity studies (Markovits et. al., Vet Path 37: 439-448, 2000), the incidence was reported at up to 24%. Similar lesions were not found in the female mice. Additionally decreased stromal polyps were described with increasing dose. The variability of granular cell tumors (based on literature search) and stromal polyps is usual for uterine findings in carcinogenicity studies. Both of these lesions are considered incidental to treatment with XP13512.

Sponsor table:

Selected Microscopic Findings: Uterus and Vagina
Terminal Necropsy
Female

Dose level (mg/kg/day)	**0**	**500**	**2000**	**5000**
Sex	**F**	**F**	**F**	**F**
Number examined	**60**	**60**	**60**	**60**
Uterus				
Granular cell tumor	1	3	3	7
Granular cell aggregate				
- mild	0	0	0	1
Stromal Polyps	11	8	2	3
Vagina				
Granular cell tumor	2	2	2	5

2.6.6.6 Reproductive and developmental toxicology

Fertility and early embryonic development

Study title: **Oral (Gavage) Fertility and General Reproductive Toxicity Study of XP13512 in Rats**

Key study findings: No significant adverse effects of dosing with XP13512 were found in mating parameters for rats treated with 0, 200, 1000 or 5000 mg/kg/d. Increased salivation was observed in the mid and high dose animals. Body weights and feed consumption were adversely affected in the high dose females immediately after the end of the dosing period (GD 8-10). They also had increased numbers of non-viable feti at necropsy. Thus, the NOAEL for reproductive toxicity in this rat study is determined to be 1000 mg/kg/d and the NOAEL for systemic toxicity is 200 mg/kg/d.

Study no.: XP035 or RD2008/00147/00 or OJA00009
Volume and page #: Electronic submission
Conducting laboratory and location: (b) (4)
Date of study initiation: 1/11/05
GLP compliance: Yes
QA reports: yes
Drug, lot #, and % purity: Lot #7 at 99.8% purity

Methods

Doses: 0 (0.1% Tween® 80 and 0.5% methylcellulose), 200, 1000 or 5000 mg/kg/d

Species/strain: Crl:CD® (SD) IGS VAF/Plus® rats

Number/sex/group: 25

Route, formulation, volume, and infusion rate: Oral gavage at 20 mL/kg; the test article solution at the mid and high doses was described as "very thick".

Satellite groups used for toxicokinetics: Not evaluated

Study design: Males were treated for 28 days prior to cohabitation and throughout the mating period. Females were treated for 15 days prior to cohabitation through Day 7 of gestation (GD7).

Parameters and endpoints evaluated: Morbidity and mortality, body weights, feed consumption, estrous cyclicity, gross necropsy, reproductive organ weights, sperm parameters, C-section parameters (corpora lutea, pregnancy status, implantation sites, viable embryos, placental appearance)

Results

Mortality: One male in each of the control, mid and high dose groups was found dead. The mid and high dose males' deaths were considered gavage accidents. One mid dose female was euthanized due to ocular damage (Day 1 of cohabitation).

Clinical signs: Males: No adverse clinical signs were observed at 200 mg/kg/d. The 1000 mg/kg/d animals showed excessive salivation. At the 5000 mg/kg/d dose, excessive salivation, soft/ liquid feces and poor grooming were described.

Females: No adverse clinical signs were observed at 200 mg/kg/d. The 1000 mg/kg/d animals showed slightly excessive salivation. At the 5000 mg/kg/d dose, excessive salivation, rales and poor grooming were described.

Body weight: Males: Treatment-related adverse effects were appreciated at 200 and 5000 mg/kg/d with decreased body weight gains and body weight loss from D 8-15. No significant adverse effects were found at 1000 mg/kg/d. The significance of the decreases at D 8-15 is uncertain due to the duration and lack of dose relationship.

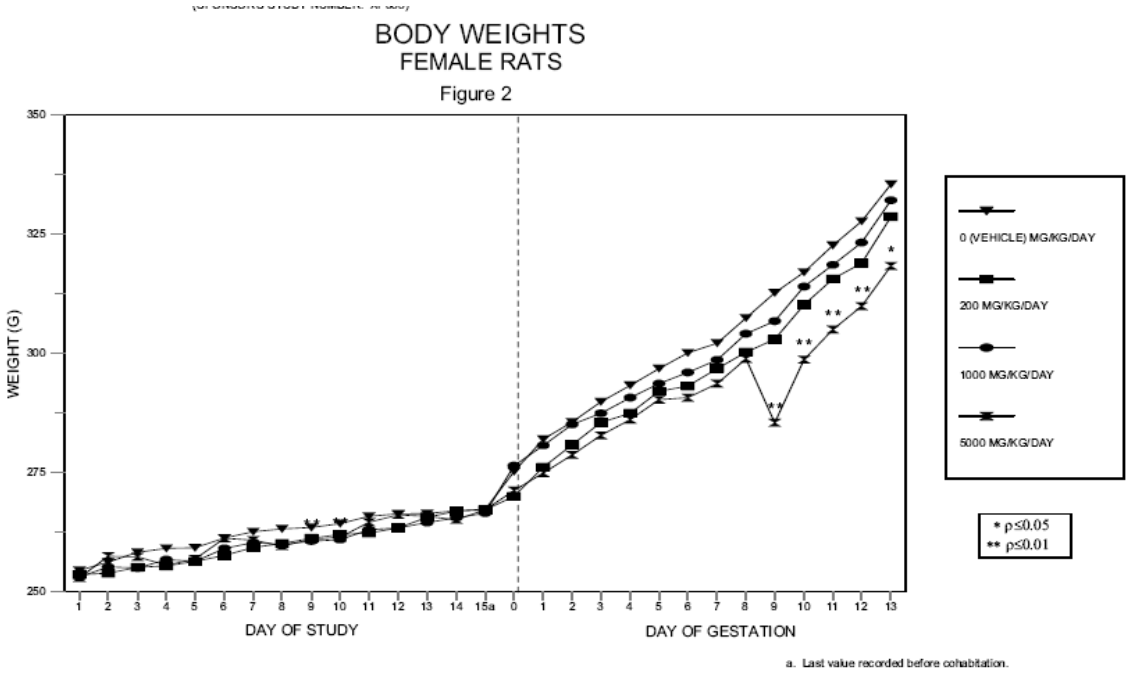
Females: From GD 8-10, significant ($p \leq 0.01$) body weight loss was determined in the 5000 mg/kg/d group. Decreased body weight gains were resultant over the immediate post-dosing period. No adverse effects were found at the lower doses.

Sponsor table and graphic:

TABLE C5 (PAGE 1): MATERNAL BODY WEIGHT CHANGES - GESTATION - SUMMARY - FEMALE RATS

DOSAGE GROUP DOSAGE (MG/KG/DAY)a		I 0 (VEHICLE)	II 200	III 1000	IV 5000
RATS TESTED	N	25	24b	25	25
PREGNANT	N	24	22	23	22
INCLUDED IN ANALYSES	N	24	22	22c	22
MATERNAL BODY WEIGHT CHANGE (G)					
DAYS 0 - 8	MEAN±S.D.	+32.2 ± 10.3	+30.2 ± 7.6	+27.7 ± 7.9	+27.6 ± 14.2
DAYS 8 - 10	MEAN±S.D.	+9.6 ± 3.8	+10.0 ± 6.2	+9.8 ± 7.5	-0.1 ± 10.0**
DAYS 10 - 13	MEAN±S.D.	+18.4 ± 6.6	+18.4 ± 6.3	+18.1 ± 9.8	+19.6 ± 4.2
DAYS 8 - 13	MEAN±S.D.	+28.0 ± 6.2	+28.4 ± 5.0	+28.0 ± 10.2	+19.5 ± 10.6**
DAYS 0 - 13	MEAN±S.D.	+60.2 ± 14.4	+58.5 ± 9.0	+55.6 ± 13.7	+47.1 ± 8.7**

DAYS = DAYS OF GESTATION
[ ] = NUMBER OF VALUES AVERAGED
a. Dosage occurred on day 1 of study through day 7 of gestation.
b. Excludes values for a rat that did not have necropsy observations or pregnancy status recorded.
c. Excludes values for rats that did not have a confirmed mating date.
** Significantly different from the vehicle control group value ($p \leq 0.01$).



Food consumption: Males: The 1000 mg/kg/d males had increased intake from D 8-22. No other differences were measured.

Females: Treatment-related decreased consumption was determined in dosed females from GD 8-10 compared to controls. However the absolute consumption was consistent during the entire post-dosing period for the low and mid dose females. Thus, the decreased consumption is only considered toxicologically significant for the high dose females.

Sponsor table:

TABLE C8 (PAGE 1): MATERNAL ABSOLUTE FEED CONSUMPTION VALUES (G/DAY) - GESTATION - SUMMARY - FEMALE RATS

DOSAGE GROUP		I	II	III	IV
DOSAGE (MG/KG/DAY)a		0 (VEHICLE)	200	1000	5000
RATS TESTED	N	25	24b	25	25
PREGNANT	N	24	22	23	22
INCLUDED IN ANALYSES	N	24	22	22c	22
MATERNAL FEED CONSUMPTION (G/DAY)					
DAYS 0 - 8	MEANtS.D.	23.7 ± 2.9	23.9 ± 2.1 [21]d	23.5 ± 2.2 [20]d,e	23.9 ± 2.2 [15]d
DAYS 8 - 10	MEANtS.D.	26.9 ± 3.4	24.2 ± 3.0*	23.7 ± 3.9* [21]e	21.0 ± 5.1**
DAYS 10 - 13	MEANtS.D.	25.0 ± 2.7	25.1 ± 2.0	24.6 ± 2.1 [21]e	24.5 ± 2.5
DAYS 8 - 13	MEANtS.D.	25.8 ± 2.7	24.7 ± 1.9	24.2 ± 2.6* [21]e	23.1 ± 2.5**
DAYS 0 - 13	MEANtS.D.	24.5 ± 2.7	24.2 ± 1.9	23.8 ± 2.1 [21]e	23.7 ± 2.2

DAYS = DAYS OF GESTATION
[ ] = NUMBER OF VALUES AVERAGED
a. Dosage occurred on day 1 of study through day 7 of gestation.
b. Excludes values for a rat that did not have necropsy observations or pregnancy status recorded.
c. Excludes values for a rat that did not have a confirmed mating date.
d. Excludes values that were associated with spillage.
e. Excludes values that were incorrectly recorded.
* Significantly different from the vehicle control group value (p≤0.05).
** Significantly different from the vehicle control group value (p≤0.01).

Necropsy: No treatment-related observations were recorded.

Fertility parameters: Males and females: No adverse effects in males on mating or fertility parameters (to include reproductive body weights or sperm parameters) were discovered.
Sponsor table:

TABLE C10 (PAGE 2): MATING AND FERTILITY, ESTROUS CYCLING AND DAYS IN COHABITATION - SUMMARY - FEMALE RATS

DOSAGE GROUP DOSAGE (MG/KG/DAY)a		I 0 (VEHICLE)	II 200	III 1000	IV 5000
MATING OBSERVATIONS					
RATS IN COHABITATION	N	25	25	25	25
INCLUDED IN ANALYSES	N	25	24b	24c	25
DAYS IN COHABITATION d	MEAN±S.D.	2.6 ± 1.4	2.2 ± 1.4	4.0 ± 4.6	2.4 ± 1.1
RATS THAT MATED	N(%)	25(100.0)	24(100.0)	24(100.0)	25(100.0)
FERTILITY INDEX e	N/N (%)	24/ 25 (96.0)	22/ 24 (91.7)	23/ 24 (95.8)	22/ 25 (88.0)
RATS WITH CONFIRMED MATING DATES	N	25	25	23	25
MATED BY FIRST MALE f DAYS 1-7	N(%)	25(100.0)	24(100.0)	21(91.3)	25(100.0)
MATED BY SECOND MALE f DAYS 15-21	N(%)	0(0.0)	0(0.0)	2(8.7)	0(0.0)
RATS PREGNANT/RATS IN COHABITATION	N/N (%)	24/ 25 (96.0)	22/ 24 (91.7)	23/ 24 (95.8)	22/ 25 (88.0)

a. Dosage occurred on day 1 of study through day 7 of presumed gestation.
b. Excludes values for a rat that did not have necropsy observations or pregnancy status recorded.
c. Excludes values for rat 3164, which was moribund sacrificed on day 16 of study.
d. Restricted to rats with a confirmed mating date and rats that did not mate.
e. Number of pregnancies/number of rats that mated.
f. Restricted to rats with a confirmed mating date.

Females: Pregnancy rates were essentially comparable across groups (96%, 91.7%, 95.8% respectively for control, low and mid dose) but lower in the high dose group (88%). There was an absolute and relative increase in non-viable embryos in the 5000 mg/kg/d females.

Sponsor tables:

TABLE C12 (PAGE 1): CAESAREAN-SECTIONING OBSERVATIONS - SUMMARY - FEMALE RATS

DOSAGE GROUP		I	II	III	IV
DOSAGE (MG/KG/DAY)a		0 (VEHICLE)	200	1000	5000
RATS TESTED	N	25	24b	24c	25
PREGNANT	N(%)	24(96.0)	22(91.7)	23(95.8)	22(88.0)
RATS PREGNANT AND CAESAREAN-SECTIONED ON DAY 13 OF GESTATION	N	24	22	23d	22
CORPORA LUTEA	MEAN±S.D.	16.7 ± 2.2	16.0 ± 2.3	16.5 ± 2.4	16.7 ± 2.1
IMPLANTATIONS	MEAN±S.D.	15.8 ± 1.7	14.5 ± 3.3	15.6 ± 2.2	15.9 ± 1.7
VIABLE EMBRYOS	N	363	305	325	318
	MEAN±S.D.	15.1 ± 1.8	13.9 ± 3.4	14.7 ± 2.2	14.4 ± 2.3
NONVIABLE EMBRYOS	N	17	15	20	31
	MEAN±S.D.	0.7 ± 1.0	0.7 ± 0.8	0.9 ± 0.9	1.4 ± 1.8
DAMS WITH VIABLE EMBRYOS	N(%)	24(100.0)	22(100.0)	22(100.0)	22(100.0)
DAMS WITH ANY NONVIABLE EMBRYOS	N(%)	11(45.8)	12(54.5)	14(60.9)	14(63.6)
DAMS WITH ALL NONVIABLE EMBRYOS	N(%)	0(0.0)	0(0.0)	0(0.0)	0(0.0)
PLACENTAE APPEARED NORMAL	N(%)	24(100.0)	22(100.0)	22(100.0)	22(100.0)
% NONVIABLE EMBRYOS/LITTER	MEAN±S.D.	4.4 ± 6.0	5.6 ± 8.0	5.6 ± 5.4	8.8 ± 11.0

a. Dosage occurred on day 1 of study through day 7 of presumed gestation.
b. Excludes values for rat 3144, which did not have necropsy observations or pregnancy status recorded.
c. Excludes values for rat 3164, which was moribund sacrificed on day 16 of study.
d. Includes values for rat 3151, which did not have a confirmed mating date.

Embryofetal development

Study title: **Oral (Gavage) Developmental Toxicity Study of XP13512 in Rats**

Key study findings: Pregnant rats were given 0, 200, 1000 or 5000 mg/kg/d of XP13512 by daily oral gavage from Gestation Day 7- 17. Maternal toxicity was appreciated at ≥1000 mg/kg/d as evidenced by excessive salivation, decreased body weights and body weight gains, and decreased feed consumption. Adverse effects were found in the feti from the high dose dams as shown by decreased fetal body weights. Neither terata nor malformations were increased by treatment. Therefore, the maternal NOAEL is 200 mg/kg/d and the fetal NOAEL is 1000 mg/kg/d in rats under the conditions of this study. The AUC and Cmax for the maternal NOAEL were 3210 µg.h/mL and 302 µg/mL, respectively, on GD 17.

Study no.: OJA00006 or RD2008/00144/00 or XP032
Volume and page #: Electronic submission
Conducting laboratory and location: (b) (4)
Date of study initiation: 1/10/05
GLP compliance: Yes
QA reports: yes
Drug, lot #, and % purity: Lot #7 at 99.8% purity

Methods

Doses: 0 (0.1% Tween® 80 + 0.5% methylcellulose), 200, 1000 or 5000 mg/kg/d These doses were selected from the dose-range finding study (#RD2007/01534/00 or XP031) where 8 pregnant Crl:CD®(SD) IGS BR VAF/Plus® rats/group were dosed at 0, 200, 500, 1500 or 5000 mg/kg/d. Adverse clinical signs (salivation, poor grooming) were found in the 5000 mg/kg/d females. Reduced body weight gains and decreased body weights were found at the high dose. Fetal body weights were decreased at this dose. Skeletal evaluations (all feti from dams ≤1500 mg/kg/d) were comparable across doses.

Species/strain: Crl:CD®(SD)IGS BR VAF/Plus® presumably pregnant rats

Number/sex/group: 25

Route, formulation, volume, and infusion rate: Oral gavage at 20 mL/kg

Satellite groups used for toxicokinetics: 6/treated group on Days 7 and 17 at 0.5, 1, 2, 4, 8, 12 and 24 hrs post-dosing (N=3/ time point)

Study design: Presumably pregnant rats were treated from GD 7- 17 daily by oral gavage. The dams were euthanized on GD 21.

Parameters and endpoints evaluated: Morbidity and mortality, body weights, feed consumption, TK, gross necropsy, C-section parameters, fetal examinations

Results

Mortality (dams): No test article-related deaths were described. One mid dose female died on the first day of dosing and was replaced.

Clinical signs (dams): Excessive salivation, primarily during the dosing period, was observed at 1000 and 5000 mg/kg/d. Poor grooming was described for the 5000 mg/kg/d dams. This sign persisted throughout the study.

Body weight (dams): No adverse effects were seen at 200 mg/kg/d. During the dosing period for the 1000 and 5000 mg/kg/d dams and during the post-dosing period for the 5000 mg/kg/d dams, significantly decreased body weights and body weight gains were measured.

Sponsor tables:

TABLE 3 (PAGE 1): MATERNAL BODY WEIGHTS - SUMMARY

DOSAGE GROUP DOSAGE (MG/KG/DAY)a		I 0(VEHICLE)	II 200	III 1000	IV 5000
RATS TESTED	N	25	25	25	25
PREGNANT	N	24	25	25	24
INCLUDED IN ANALYSES	N	23b	25	25	24
MATERNAL BODY WEIGHT (G)					
DAY 0	MEAN±S.D.	234.3 ± 9.3	233.2 ± 9.2	233.7 ± 9.7	233.2 ± 9.3
DAY 7	MEAN±S.D.	274.2 ± 16.3	273.8 ± 15.5	274.1 ± 11.8	270.9 ± 15.3
DAY 8	MEAN±S.D.	278.1 ± 15.7	277.1 ± 15.4	273.2 ± 11.5	269.7 ± 17.5
DAY 9	MEAN±S.D.	283.2 ± 16.6	281.9 ± 16.8	276.6 ± 10.4	267.7 ± 17.8**
DAY 10	MEAN±S.D.	288.5 ± 16.4	285.8 ± 18.0	279.0 ± 13.4	271.8 ± 16.2**
DAY 11	MEAN±S.D.	295.4 ± 17.2	291.1 ± 18.8	285.0 ± 12.1*	278.1 ± 16.9**
DAY 12	MEAN±S.D.	300.4 ± 17.9	295.9 ± 19.4	290.9 ± 14.0	284.4 ± 17.0**
DAY 13	MEAN±S.D.	308.0 ± 16.8	301.9 ± 19.2	296.7 ± 14.5*	289.2 ± 17.4**
DAY 14	MEAN±S.D.	313.2 ± 19.4	305.8 ± 20.3	300.5 ± 15.3*	294.0 ± 16.0**
DAY 15	MEAN±S.D.	318.8 ± 18.1	312.3 ± 21.8	309.1 ± 16.7	302.9 ± 17.9**
DAY 16	MEAN±S.D.	328.3 ± 21.4	321.6 ± 22.7	316.3 ± 15.5*	308.5 ± 19.1**
DAY 17	MEAN±S.D.	342.7 ± 21.6	334.2 ± 22.5	330.0 ± 15.6*	321.1 ± 19.8**

DAY = DAY OF GESTATION
a. Dosage occurred on days 7 through 17 of gestation.
b. Excludes values for dam 3823; the litter consisted of 11 early resorptions.
* Significantly different from the vehicle control group value (p≤0.05).
** Significantly different from the vehicle control group value (p≤0.01).

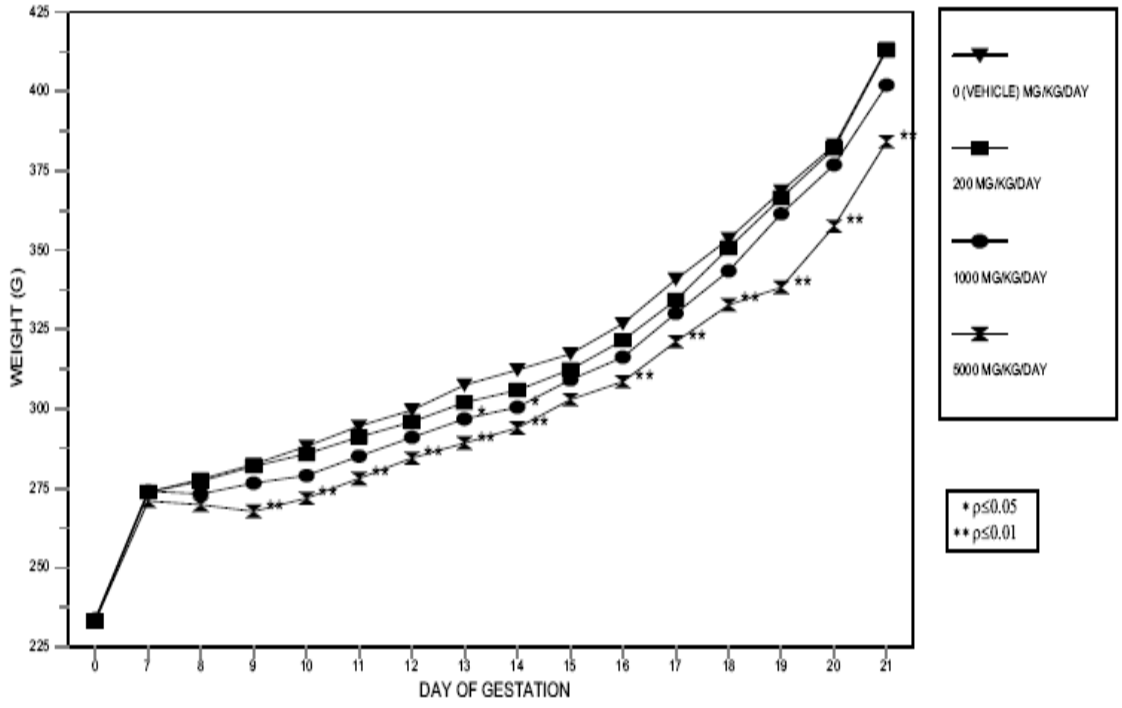
TABLE 3 (PAGE 2): MATERNAL BODY WEIGHTS - SUMMARY

DOSAGE GROUP DOSAGE (MG/KG/DAY)a		I 0(VEHICLE)	II 200	III 1000	IV 5000
RATS TESTED	N	25	25	25	25
PREGNANT	N	24	25	25	24
INCLUDED IN ANALYSES	N	23b	25	25	24
MATERNAL BODY WEIGHT (G)					
DAY 18	MEAN±S.D.	356.2 ± 23.4	350.7 ± 24.9	343.4 ± 15.7*	332.7 ± 16.2**
DAY 19	MEAN±S.D.	371.6 ± 24.8	366.4 ± 25.2	361.4 ± 15.6	338.2 ± 19.8**
DAY 20	MEAN±S.D.	386.6 ± 26.7	382.0 ± 26.4	376.9 ± 18.9	357.6 ± 20.9**
DAY 21	MEAN±S.D.	417.9 ± 31.2	412.9 ± 31.5	401.9 ± 22.8	384.2 ± 25.0**

DAY = DAY OF GESTATION
a. Dosage occurred on days 7 through 17 of gestation.
b. Excludes values for dam 3823; the litter consisted of 11 early resorptions.
* Significantly different from the vehicle control group value ($p \leq 0.05$).
** Significantly different from the vehicle control group value ($p \leq 0.01$).

MATERNAL BODY WEIGHTS

Figure 1



Food consumption (dams): As for body weights, no adverse effects were seen at 200 mg/kg/d. At the mid dose, decreased consumption was determined for the post-dosing period. The high dose dams had decreased consumption throughout the study period.

TABLE 5 (PAGE 1): MATERNAL ABSOLUTE FEED CONSUMPTION VALUES (G/DAY) - SUMMARY

DOSAGE GROUP		I	II	III	IV
DOSAGE (MG/KG/DAY)a		0(VEHICLE)	200	1000	5000
RATS TESTED	N	25	25	25	25
PREGNANT	N	24	25	25	24
INCLUDED IN ANALYSES	N	23b	25	25	24
MATERNAL FEED CONSUMPTION (G/DAY)					
DAYS 0 - 7	MEAN±S.D.	22.8 ± 2.5	23.1 ± 2.3 [23]c	23.4 ± 1.9	22.5 ± 2.9
DAYS 7 - 10	MEAN±S.D.	24.7 ± 2.6	24.6 ± 3.1	23.8 ± 3.0 [24]c	19.3 ± 4.3**
DAYS 10 - 12	MEAN±S.D.	26.1 ± 2.4	25.8 ± 3.2	26.4 ± 5.4	23.0 ± 3.2**
DAYS 12 - 15	MEAN±S.D.	25.3 ± 2.3	24.2 ± 4.1	25.0 ± 2.8	23.0 ± 3.1
DAYS 15 - 18	MEAN±S.D.	25.8 ± 5.0	26.1 ± 3.4	26.6 ± 2.5	24.4 ± 2.8 [23]c
DAYS 7 - 18	MEAN±S.D.	25.4 ± 2.1	25.1 ± 2.8	25.4 ± 1.9	22.4 ± 2.8** [23]c
DAYS 18 - 21	MEAN±S.D.	28.5 ± 3.4 [22]c	26.4 ± 2.2* [23]c	25.7 ± 4.8** [24]c	24.2 ± 2.8**
DAYS 7 - 21	MEAN±S.D.	26.1 ± 2.2 [22]c	25.3 ± 2.6 [23]c	25.5 ± 1.9 [24]c	22.7 ± 2.3**
DAYS 0 - 21	MEAN±S.D.	25.0 ± 2.2 [22]c	24.7 ± 2.4 [23]c	24.9 ± 1.4 [24]c	22.7 ± 2.3**

DAYS = DAYS OF GESTATION
[ ] = NUMBER OF VALUES AVERAGED
a. Dosage occurred on days 7 through 17 of gestation.
b. Excludes values for dam 3823; the litter consisted of 11 early resorptions.
c. Excludes values that were associated with spillage.
* Significantly different from the vehicle control group value ($p \leq 0.05$).
** Significantly different from the vehicle control group value ($p \leq 0.01$).

Toxicokinetics: Exposures to gabapentin increased with increasing doses and some accumulation was found with AUCs slightly higher on GD 17. Very low levels of XP13512 were found at any of the time points.
Sponsor table:

Whole Blood Exposure of XP13512 and Gabapentin after Oral Administration of XP13512 in Pregnant Rats

Pharmacokinetic Parameter	XP13512 Dosage (mg/kg/day)	Gabapentin		XP13512	
		DG 7	DG 17	DG 7	DG 17
AUC (µg·hr/mL)	200	148	195	1.28	1.33
	1000	757	988	5.91	8.20
	5000	3210*	3310	26.9*	31.9
C_{max} (µg/mL)	200	44.4	55.5	0.595	0.758
	1000	130	166	1.39	1.54
	5000	226	302	1.86	12.8
T_{max} (hr)	200	1.00	1.00	0.500	0.500
	1000	1.00	1.00	0.500	0.500
	5000	4.00	8.00	4.00	0.500

AUC = Area under the concentration vs. time curve ($AUC_{(0-infinity)}$ on DG 7 and $AUC_{(0-24)}$ on DG 17)
C_{max} = Concentration maximum; T_{max} = Time to C_{max}.
*$AUC_{(last)}$ since $AUC_{(0-infinity)}$ was probably artifactually high.

Terminal and necroscopic evaluations: Neither gross necropsy nor C-section parameters were adversely affected by treatment with XP13512. Pregnancy rates were comparable across groups (96%, 100%, 100% and 96%, respectively). One control dam had only resorbed feti and her information was not utilized in the summary tables.

Offspring: Decreased fetal weights, both male and total, were decreased for the high dose feti. In the mid and high dose groups, the percentages, but not the actual number, of dead or resorbed feti were significantly increased. No other adverse effects of dosing were found and fetal ossification sites were comparable across groups.

Sponsor table:

TABLE 8 (PAGE 1): LITTER OBSERVATIONS (CAESAREAN-DELIVERED FETUSES) - SUMMARY

DOSAGE GROUP		I	II	III	IV
DOSAGE (MG/KG/DAY)a		0(VEHICLE)	200	1000	5000
LITTERS WITH ONE OR MORE LIVE FETUSES	N	23	25	25	24
IMPLANTATIONS	MEAN±S.D.	14.9 ± 1.8	15.4 ± 1.6	15.2 ± 1.7	14.7 ± 2.4
LIVE FETUSES	N	339	363	351	328
	MEAN±S.D.	14.7 ± 1.7	14.5 ± 2.4	14.0 ± 3.2	13.7 ± 2.0
LIVE MALE FETUSES	N	164	191	177	178
% LIVE MALE FETUSES/LITTER	MEAN±S.D.	48.2 ± 14.0	52.8 ± 13.7	52.2 ± 19.2	55.0 ± 13.8
LIVE FETAL BODY WEIGHTS (GRAMS)/LITTER	MEAN±S.D.	5.16 ± 0.35	5.22 ± 0.30	5.23 ± 0.41	4.93 ± 0.36*
MALE FETUSES	MEAN±S.D.	5.31 ± 0.35	5.38 ± 0.30	5.37 ± 0.40	5.03 ± 0.36*
FEMALE FETUSES	MEAN±S.D.	5.03 ± 0.38	5.04 ± 0.32	5.01 ± 0.25 [24]b	4.80 ± 0.40
% DEAD OR RESORBED CONCEPTUSES/LITTER	MEAN±S.D.	1.1 ± 3.1	5.5 ± 11.4	7.5 ± 18.5*	6.1 ± 9.2*

[ ] = NUMBER OF VALUES AVERAGED
a. Dosage occurred on days 7 through 17 of gestation.
b. Litter 3858 had no female fetuses.
* Significantly different from the vehicle control group value ($p \leq 0.05$).

Study title: **Oral (Stomach Tube) Developmental Toxicity Study of XP13512 in Rabbits**

Key study findings: XP13512 was administered daily to presumably pregnant rabbits at 0, 200, 500 or 2500 mg/kg/d. Maternal toxicity as evidenced by poor grooming and scant/soft/liquid feces in addition to hypoactivity and ataxia were observed at 2500 mg/kg/d. Individual rabbits from this dose group also lost their righting reflex. Decreased feed consumption and body weight losses were reported in the mid and high dose does. Premature delivery and/or abortion attributable to dosing (adverse clinical signs, decreased feed consumption and body weight loss were seen prior to the events) were noted in the high dose group.

Under the conditions of this embryo-fetal study in rabbits, the maternal NOAEL is determined to be 200 mg/kg/d and the fetal NOAEL is 2500 mg/kg/d (highest dose tested). The AUC and Cmax at the maternal NOAEL were 311 µg.h/mL and 105µg/mL, respectively, on GD 19.

Study no.: OJA00008 or RD2008/00146/00 or XP034
Volume and page #: Electronic submission
Conducting laboratory and location: (b) (4)
Date of study initiation: 1/31/05
GLP compliance: Yes
QA reports: yes

Drug, lot #, and % purity: Lot #7 at 99.8% purity

Methods

Doses: 0 (0.1% Tween® 80 + 0.5% methylcellulose), 200, 500 or 2500 mg/kg/d These doses were selected from the dose-range finding study (#RD2008/00145/00 or XP033) where 5 pregnant Hra: (NZW)SPF rabbits/group were dosed at 0, 200, 500, 1500 or 5000 mg/kg/d. Adverse clinical signs (hypoactivity, ataxia, poor grooming) were found at ≥500 mg/kg/d and soft/liquid feces were found additionally in the 5000 mg/kg/d females. Reduced body weight gains and decreased body weights were found at the 1500 and 5000 mg/kg/d dose. Fetal body weights were decreased at 5000 mg/kg/d. Skeletal evaluations (all feti from dams ≤1500 mg/kg/d) were comparable across doses.

Species/strain: Hra: (NZW)SPF time-mated rabbits
Number/sex/group: 20
Route, formulation, volume, and infusion rate: Via stomach tube at 20 mL/kg
Satellite groups used for toxicokinetics: 3/group euthanized on GD 20 after the last samples were taken
Study design: Does were treated from GD 7-19. On GD 29, the survivors were euthanized and examined.
Parameters and endpoints evaluated: Morbidity and mortality, body weight, feed consumption, TK (Days 7 and 19 at 0.5, 1, 2, 4, 8, 12 and 24 hrs post-dosing) from the satellite animals, gross necropsy, C-section parameters, fetal parameters

Results

Mortality (dams): Main study: Gavage accidents accounted for premature deaths of 2 controls (#408, GD 12 and #420, GD 10) 1 at 200 mg/kg/d (#424, GD 10) and 1 at 2500 mg/kg/d (#477, GD 16).

TK study: One high dose dam (#489) was found dead on GD10 after showing ataxia on GD 7 and 8, hypoactivity on GD 9. Her death was attributed to a gavage accident.

Clinical signs (dams): Main study: No adverse effects were appreciated at 200 mg/kg/d. At the high dose, poor grooming and scant/soft/liquid feces in addition to hypoactivity (9 does) and ataxia (7 does) were observed. An individual rabbit from this dose group also lost their righting reflex.

Abortions were observed in 1 control (#415, GD 22), 1 mid dose (#444, GD 27) and 2 high dose (#462, GD 25; #469, GD 27 [probable gavage accident]) animals after the end of the dosing period. These animals were sacrificed after they aborted. One of the high dose dam's abortions was attributed to an earlier gavage error based on the necropsy findings. There was one premature delivery on GD 29 from the high dose (#471). This doe had previously been ataxic and hypoactive, had scant feces and an unkempt coat. Both the abortion and the premature delivery in the high dose animals were considered to be due to treatment with XP13512 as they'd shown decreased feed consumption and body weight loss from GD 7-9 as well as adverse clinical signs (scant feces beginning on GD 14, unkempt coat) prior to their abortions/ deliveries. Two of these animals had

tricobezoars in their stomachs at necropsy which may have contributed to their adverse clinical presentation.

TK study: According to the sponsor, clinical signs similar to those noted in the main study does were observed in the TK does as well.

Body weight and food consumption (dams): No adverse effects on feed consumption were found at the low dose. Decreased consumption and body weight loss were noted in the 500 mg/kg/d does on GD 20-24. In the 2500 mg/kg/d group, decreased consumption was reported during the entire study and body weight losses occurred from GD 13-24.

Sponsor table and figure:

MATERNAL BODY WEIGHTS

Figure 1

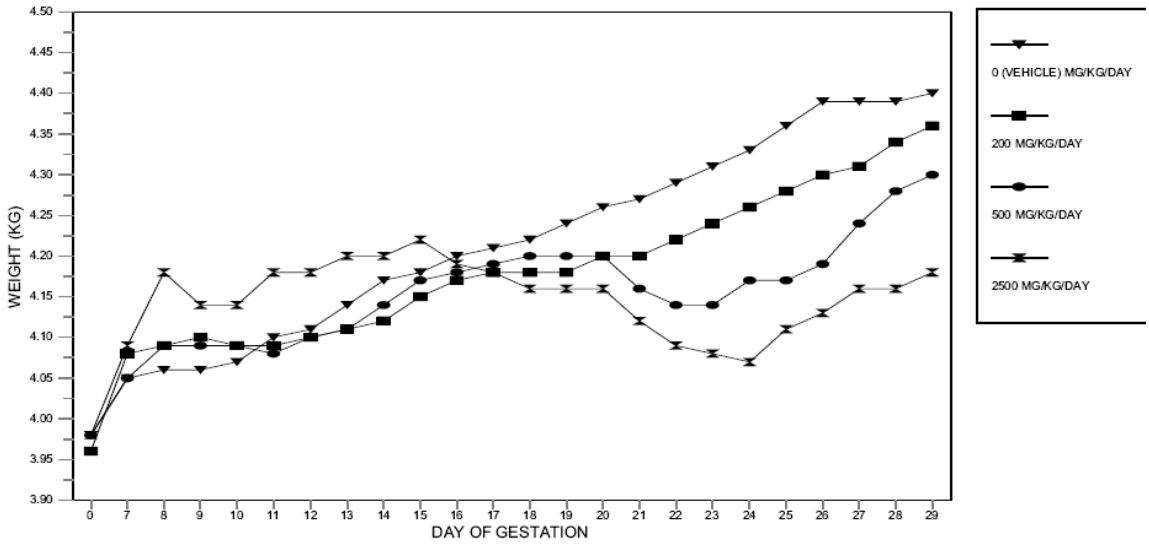


TABLE 4 (PAGE 1): MATERNAL BODY WEIGHTS - SUMMARY

DOSAGE GROUP DOSAGE (MG/KG/DAY)a		I 0(VEHICLE)	II 200	III 500	IV 2500
RABBITS TESTED	N	20	20	20	20
PREGNANT	N	20	19	20	20
MATERNAL BODY WEIGHT (KG)					
DAY 0	MEAN±S.D.	3.98 ± 0.33	3.96 ± 0.33	3.98 ± 0.29	3.98 ± 0.34
DAY 7	MEAN±S.D.	4.05 ± 0.32	4.08 ± 0.29	4.05 ± 0.27	4.09 ± 0.33
DAY 8	MEAN±S.D.	4.06 ± 0.31	4.09 ± 0.28	4.09 ± 0.28	4.18 ± 0.37
DAY 9	MEAN±S.D.	4.06 ± 0.33	4.10 ± 0.29	4.09 ± 0.28	4.14 ± 0.37
DAY 10	MEAN±S.D.	4.07 ± 0.34	4.09 ± 0.30	4.09 ± 0.28	4.14 ± 0.35
DAY 11	MEAN±S.D.	4.10 ± 0.29 [19]b	4.09 ± 0.30	4.08 ± 0.27	4.18 ± 0.37
DAY 12	MEAN±S.D.	4.11 ± 0.31 [19]b	4.10 ± 0.30	4.10 ± 0.29	4.18 ± 0.36
DAY 13	MEAN±S.D.	4.14 ± 0.33 [18]b	4.11 ± 0.30	4.11 ± 0.28	4.20 ± 0.38
DAY 14	MEAN±S.D.	4.17 ± 0.34 [18]b	4.12 ± 0.31 [18]b	4.14 ± 0.28	4.20 ± 0.37
DAY 15	MEAN±S.D.	4.18 ± 0.35 [18]b	4.15 ± 0.32 [18]b	4.17 ± 0.28	4.22 ± 0.37
DAY 16	MEAN±S.D.	4.20 ± 0.32 [18]b	4.17 ± 0.32 [18]b	4.18 ± 0.29	4.19 ± 0.37
DAY 17	MEAN±S.D.	4.21 ± 0.31 [18]b	4.18 ± 0.34 [18]b	4.19 ± 0.29	4.18 ± 0.37 [19]b
DAY 18	MEAN±S.D.	4.22 ± 0.30 [18]b	4.18 ± 0.34 [18]b	4.20 ± 0.30	4.16 ± 0.37 [19]b
DAY 19	MEAN±S.D.	4.24 ± 0.31 [18]b	4.18 ± 0.34 [18]b	4.20 ± 0.29	4.16 ± 0.40 [19]b
DAY 20	MEAN±S.D.	4.26 ± 0.30 [18]b	4.20 ± 0.34 [18]b	4.20 ± 0.30	4.16 ± 0.39 [19]b
DAY 21	MEAN±S.D.	4.27 ± 0.32 [18]b	4.20 ± 0.35 [18]b	4.16 ± 0.31	4.12 ± 0.38 [19]b

DAY = DAY OF GESTATION
[ ] = NUMBER OF VALUES AVERAGED
a. Dosage occurred on days 7 through 19 of gestation.
b. Excludes values for rabbits that aborted or were found dead.

TABLE 5 (PAGE 1): MATERNAL BODY WEIGHT CHANGES - SUMMARY

DOSAGE GROUP DOSAGE (MG/KG/DAY)a		I 0(VEHICLE)	II 200	III 500	IV 2500
RABBITS TESTED	N	20	20	20	20
PREGNANT	N	20	19	20	20
MATERNAL BODY WEIGHT CHANGE (KG)					
DAYS 0 - 7	MEAN±S.D.	+0.08 ± 0.08	+0.12 ± 0.07	+0.07 ± 0.09	+0.12 ± 0.07
DAYS 7 - 10	MEAN±S.D.	+0.02 ± 0.07	+0.01 ± 0.05	+0.04 ± 0.06	+0.05 ± 0.10
DAYS 10 - 13	MEAN±S.D.	+0.03 ± 0.05 [18]b	+0.02 ± 0.07	+0.02 ± 0.04	+0.06 ± 0.07
DAYS 13 - 16	MEAN±S.D.	+0.06 ± 0.05 [18]b	+0.07 ± 0.05 [18]b	+0.07 ± 0.04	-0.01 ± 0.08**
DAYS 16 - 20	MEAN±S.D.	+0.06 ± 0.07 [18]b	+0.04 ± 0.07 [18]b	+0.02 ± 0.06	-0.04 ± 0.08** [19]b
DAYS 7 - 20	MEAN±S.D.	+0.18 ± 0.09 [18]b	+0.13 ± 0.13 [18]b	+0.15 ± 0.08	+0.07 ± 0.20 [19]b
DAYS 20 - 24	MEAN±S.D.	+0.08 ± 0.06 [17]b	+0.06 ± 0.08 [18]b	-0.03 ± 0.11**	-0.09 ± 0.08** [19]b
DAYS 24 - 29	MEAN±S.D.	+0.07 ± 0.10 [17]b	+0.10 ± 0.07 [18]b	+0.11 ± 0.09 [19]b	+0.05 ± 0.13 [17]b
DAYS 20 - 29	MEAN±S.D.	+0.15 ± 0.11 [17]b	+0.16 ± 0.11 [18]b	+0.08 ± 0.13 [19]b	-0.03 ± 0.15** [17]b
DAYS 7 - 29	MEAN±S.D.	+0.32 ± 0.12 [17]b	+0.29 ± 0.16 [18]b	+0.24 ± 0.16 [19]b	+0.09 ± 0.23** [17]b
DAYS 0 - 29	MEAN±S.D.	+0.40 ± 0.15 [17]b	+0.40 ± 0.19 [18]b	+0.30 ± 0.20 [19]b	+0.21 ± 0.26** [17]b

DAYS = DAYS OF GESTATION
[ ] = NUMBER OF VALUES AVERAGED
a. Dosage occurred on days 7 through 19 of gestation.
b. Excludes values for rabbits that aborted, prematurely delivered or were found dead.
** Significantly different from the vehicle control group value (p≤0.01).

TK study: According to the sponsor, maternal consumption and body weight effects were comparable to those in the main study.

Toxicokinetics: Exposure to gabapentin increased with increasing doses but, unlike the rat study, exposures were slightly lower on GD 19 compared to GD 7. Exposures to XP13512 were consistently low although slightly higher on GD 19 than on GD 7.
Sponsor table:

Whole Blood Exposure of XP13512 and Gabapentin after Oral Administration of XP13512 in Pregnant Rabbits

Pharmacokinetic Parameter	XP13512 Dosage (mg/kg/day)	Gabapentin		XP13512	
		DG 7	DG 19	DG 7	DG 19
AUC (µg·hr/mL)	200	357	311	0.970	1.40
	500	1000	944	0.963	1.57
	2500	4980	3040	2.69	3.31
C_{max} (µg/mL)	200	94.8	105	0.194	0.126
	500	195	253	0.303	0.255
	2500	392	393	0.857	0.879
T_{max} (hr)	200	0.500	0.500	0.500	0.500
	500	0.500	0.667	0.500	0.500
	2500	1.33	2.50	0.500	0.500

AUC = Area under the concentration vs. time curve [$AUC_{(0\text{-infinity})}$ on DG 7 and $AUC_{(0\text{-}24)}$ on DG 19]
C_{max} = Concentration maximum
T_{max} = Time to C_{max}

Terminal and necroscopic evaluations: No gross necropsy lesions were found that were attributable to treatment. Pregnancy rates were comparable across groups with C-section data provided for 17, 18, 19 and 16 does found pregnant at necropsy and the results were comparable across the control, low and mid dose groups. Fetal body weights were reduced in females and there was a decrease in implantations and live fetuses from the high dose group.

Sponsor table:

TABLE 9 (PAGE 1): LITTER OBSERVATIONS (CAESAREAN-DELIVERED FETUSES) - SUMMARY

DOSAGE GROUP		I	II	III	IV
DOSAGE (MG/KG/DAY)a		0(VEHICLE)	200	500	2500
LITTERS WITH ONE OR MORE LIVE FETUSES	N	17	18	19	16
IMPLANTATIONS	MEAN±S.D.	9.8 ± 1.9	9.0 ± 2.6	8.9 ± 2.9	7.9 ± 2.2
LIVE FETUSES	N	162	159	160	121
	MEAN±S.D.	9.5 ± 2.3	8.8 ± 2.4	8.4 ± 2.5	7.6 ± 2.2
LIVE MALE FETUSES	N	83	74	86	59
% LIVE MALE FETUSES/LITTER	MEAN±S.D.	51.3 ± 10.4	46.5 ± 20.6	53.6 ± 23.3	47.8 ± 18.8
LIVE FETAL BODY WEIGHTS (GRAMS)/LITTER	MEAN±S.D.	43.35 ± 3.30	44.64 ± 5.41	43.74 ± 6.85	40.60 ± 7.26
MALE FETUSES	MEAN±S.D.	43.36 ± 3.99	44.82 ± 5.20 [17]b	44.19 ± 7.49	42.22 ± 8.62
FEMALE FETUSES	MEAN±S.D.	43.76 ± 3.85	44.43 ± 5.81	42.40 ± 6.17 [17]c	37.93 ± 8.53*
% RESORBED CONCEPTUSES/LITTER	MEAN±S.D.	4.1 ± 12.2	1.9 ± 5.4	4.8 ± 7.7	4.4 ± 8.5

[ ] = NUMBER OF VALUES AVERAGED
a. Dosage occurred on days 7 through 19 of gestation.
b. Litter 428 had no male fetuses.
c. Litter 441 and 452 had no female fetuses.
* Significantly different from the vehicle control group value (p≤0.05).

Offspring: No significant increases in either malformations or variations were found in any dose group when compared to controls. Fetal ossification sites were comparable across dose groups. Decreased body weights of the female feti (N=2) from Doe #461 in the 2500 mg/kg/d group but not in the single male from the litter. These values were excluded from the litter calculations.

Sponsor table:

TABLE 10 (PAGE 1): FETAL ALTERATIONS - SUMMARY

DOSAGE GROUP		I	II	III	IV
DOSAGE (MG/KG/DAY)a		0(VEHICLE)	200	500	2500
LITTERS EVALUATED	N	17	18	19	16
FETUSES EVALUATED	N	162	159	160	121
LIVE	N	162	159	160	121
LITTERS WITH FETUSES WITH ANY ALTERATION OBSERVED	N(%)	6(35.3)	11(61.1)	11(57.9)	10(62.5)
FETUSES WITH ANY ALTERATION OBSERVED	N(%)	14(8.6)	17(10.7)	21(13.1)	14(11.6)
% FETUSES WITH ANY ALTERATION/LITTER	MEAN±S.D.	11.6 ± 24.8	10.0 ± 9.9	15.2 ± 16.3	12.5 ± 11.8

a. Dosage occurred on days 7 through 19 of gestation.

Prenatal and postnatal development

Study title: **Oral (Gavage) Developmental and Perinatal/ Postnatal Reproduction Toxicity Study of XP13512 in Rats, Including a Postnatal Behavioral/ Functional Evaluation**

Key study findings: Pregnant CD rats were treated with XP13512 at 0, 200, 1000 or 5000 mg/kg/d from GD 7- LD 20. As in previously reviewed studies, adverse effects on clinical signs, body weights and feed consumption were seen at ≥1000 mg/kg/d. Viability and fetal growth were adversely affected at the same doses. No effects on the reproductive capacity or outcome of the pregnancies of the F1 generation were seen at any dose. The NOAEL for maternal effects is determined to be 200 mg/kg/d and the reproductive NOEL is 5000 mg/kg/d.

Study no.: OJA00010 or RD2008/00148/00 or XP036
Volume and page #: Electronic submission
Conducting laboratory and location: (b) (4)
Date of study initiation: 4/3/05
GLP compliance: Yes
QA reports: yes
Drug, lot #, and % purity: Lot #7 at 99.8% purity

Methods

Doses: 0 (0.1% Tween® 80 + 0.5% methylcellulose), 200, 1000, or 5000 mg/kg/d

Species/strain: Crl: CD(SD) rats

Number/sex/group: 25 presumably pregnant rats

Route, formulation, volume, and infusion rate: Oral gavage at 20 mL/kg

Satellite groups used for toxicokinetics: None

Study design: The dams were treated from GD 7- Lactation Day (LD) 20 or if not pregnant, GD7 -24. Untreated F1 pups selected for breeding were bred at approximately 90 days of age. Males were euthanized and females were continued until C-section on GD 21. Their feti were examined.

Parameters and endpoints evaluated: Morbidity and mortality, clinical signs, body weights, feed consumption, parturition, litter parameters, pup viability, maternal behavior, gross necropsy of dams on LD 21 as well as pups not selected for reproduction; F1 pups: Clinical observations, body weights, feed consumption, sexual maturation, learning and memory (Day 24: utilized a passive avoidance apparatus with dark and light compartments; Day 70: a water-filled M-maze was used to measure overt coordination, swimming ability as well as learning and memory), C-section parameters, fetal parameters

Results

F_0 in-life: One control female (#8120) had dystocia on GD 23 (3 late resorptions, 11 early resorptions) and was sacrificed. One high dose female (#8196) experienced a gavage accident and was sacrificed on LD 20; a perforated esophagus was detected at necropsy.

No adverse clinical effects were reported in the 200 mg/kg/d group during gestation or lactation. The 1000 mg/kg/d dams showed decreased body weight gains from GD 7-10 but no effect on feed consumption was appreciated. During the lactation period, these dams had body weight loss and decreased consumption early on and lower body weights during the entire period and some dams evidenced excessive salivation. Dams from the 5000 mg/kg/d group had body weight loss early in the dosing period and had continued to demonstrate decreased feed consumption and body weight decrements during gestation and lactation as well as excessive salivation and unkempt coats.

Sponsor table:

TABLE B4 (PAGE 1): MATERNAL BODY WEIGHT CHANGES - GESTATION - SUMMARY - F0 GENERATION FEMALE RATS

DOSAGE GROUP DOSAGE (MG/KG/DAY)a		I 0 (VEHICLE)	II 200	III 1000	IV 5000
RATS TESTED	N	25	25	25	25
PREGNANT	N	24	24	25	25
MATERNAL BODY WEIGHT CHANGE (G)					
DAYS 0 - 7	MEAN±S.D.	+38.6 ± 7.2	+37.4 ± 6.3	+38.6 ± 6.8	+36.1 ± 5.8
DAYS 7 - 10	MEAN±S.D.	+15.5 ± 3.7	+13.4 ± 4.8	+10.3 ± 5.1**	-2.1 ± 12.4**
DAYS 10 - 12	MEAN±S.D.	+14.0 ± 3.9	+12.0 ± 4.0	+12.4 ± 3.9	+12.3 ± 8.2
DAYS 12 - 15	MEAN±S.D.	+22.5 ± 5.0	+21.9 ± 5.6	+21.1 ± 7.2	+21.2 ± 6.4
DAYS 15 - 18	MEAN±S.D.	+37.2 ± 6.4	+36.7 ± 5.1	+38.1 ± 8.4	+33.3 ± 9.0
DAYS 18 - 20	MEAN±S.D.	+27.0 ± 10.4	+27.2 ± 5.6	+29.8 ± 8.0	+26.0 ± 8.3
DAYS 7 - 20	MEAN±S.D.	+116.3± 15.7	+111.3± 14.8	+111.7± 19.0	+90.7 ± 22.2**
DAYS 0 - 20	MEAN±S.D.	+154.9± 17.4	+148.7± 16.6	+150.3± 22.1	+126.8± 25.0**

DAYS = DAYS OF GESTATION
a. Dosage occurred on day 7 of gestation through day 20 of lactation.
** Significantly different from the vehicle control group value (p≤0.01).

Pregnancy rates (24, 24, 25 and 25 dams were pregnant in the respective dose groups) and gestation lengths were toxicologically comparable across groups. Although the duration of gestation was increased in the high dose dams, the duration (22 or 23 days) was within the historical control range for the testing laboratory. Thus, the rates are considered to be comparable across groups. Unkempt coats were observed in the high dose pups (9 litters), presumably due to poor maternal caretaking. Pup weights were decreased on LD 7 in the 1000 and 5000 mg/kg/d groups and for the remainder of the lactation period for the high dose pups (sponsor's table below). Pup mortality was increased in the high dose group (3, 5, 5 and 20 pups for the respective dose groups).

TABLE B11 (PAGE 1): NATURAL DELIVERY OBSERVATIONS - SUMMARY - F0 GENERATION FEMALE RATS

DOSAGE GROUP DOSAGE (MG/KG/DAY)a		I 0 (VEHICLE)	II 200	III 1000	IV 5000
RATS ASSIGNED TO NATURAL DELIVERY	N	25	25	25	25
PREGNANT	N	24	24	25	25
DELIVERED A LITTER	N(%)	23(95.8)	24(100.0)	25(100.0)	25(100.0)
DURATION OF GESTATION b	MEAN±S.D.	22.6 ± 0.5	22.7 ± 0.5	22.9 ± 0.3	23.0 ± 0.2**
IMPLANTATION SITES PER DELIVERED LITTER	N MEAN±S.D.	332 14.4 ± 2.0	348 14.5 ± 2.1	358 14.3 ± 2.3	353 14.1 ± 2.2
DAMS WITH STILLBORN PUPS	N(%)	1(4.3)	4(16.7)	2(8.0)	3(12.0)
DAMS WITH NO LIVEBORN PUPS	N(%)	0(0.0)	0(0.0)	0(0.0)	0(0.0)
GESTATION INDEX c	% N/N	95.8 23/ 24	100.0 24/ 24	100.0 25/ 25	100.0 25/ 25
DAMS WITH ALL PUPS DYING DAYS 1-4 POSTPARTUM	N(%)	0(0.0)	0(0.0)	0(0.0)	0(0.0)
DAMS WITH ALL PUPS DYING DAYS 5-21 POSTPARTUM	N(%)	0(0.0)	0(0.0)	0(0.0)	0(0.0)

a. Dosage occurred on day 7 of gestation through day 20 of lactation.
b. Calculated as the time (in days) elapsed between confirmed mating (arbitrarily defined as day 0) and the time (in days) the first pup was delivered.
c. Number of rats with live offspring/number of pregnant rats.
** Significantly different from the vehicle control group value (p≤0.01).

TABLE B12 (PAGE 1): LITTER OBSERVATIONS (NATURALLY DELIVERED PUPS) - SUMMARY - F1 GENERATION LITTERS

DOSAGE GROUP DOSAGE (MG/KG/DAY)a		I 0 (VEHICLE)	II 200	III 1000	IV 5000
DELIVERED LITTERS WITH ONE OR MORE LIVEBORN PUPS	N	23	24	25	25
PUPS DELIVERED (TOTAL)	N MEAN±S.D.	308 13.4 ± 2.3	326 13.6 ± 2.1	347 13.9 ± 2.4	333 13.3 ± 2.5
LIVEBORN	MEAN±S.D. N(%)	13.2 ± 2.1 304(98.7)	13.3 ± 2.1 320(98.2)	13.8 ± 2.2 343(98.8)	13.1 ± 2.5 328(98.5)
STILLBORN	MEAN±S.D. N(%)	0.2 ± 0.8 4(1.3)	0.2 ± 0.6 6(1.8)	0.1 ± 0.4 4(1.2)	0.2 ± 0.6 5(1.5)
PUPS FOUND DEAD OR PRESUMED CANNIBALIZED					
DAY 1	N/N(%)	1/304(0.3)	2/320(0.6)	2/344(0.6)	4/328(1.2)
DAYS 2- 4	N/N(%)	1/303(0.3)	5/318(1.6)	3/342(0.9)	19/324(5.9)**
DAYS 5- 7	N/N(%)	0/302(0.0)	3/312(1.0)b	1/339(0.3)	3/305(1.0)
DAYS 8-14	N/N(%)	2/302(0.7)	2/309(0.6)	8/338(2.4)**	1/302(0.3)
DAYS 15-21	N/N(%)	0/300(0.0)	0/307(0.0)	0/330(0.0)	9/288(3.1)**c
VIABILITY INDEX d	% N/N	99.3 302/304	97.8 313/320	98.5 339/344	93.0 305/328**
LACTATION INDEX e	% N/N	99.3 300/302	98.4b 307/312b	97.3 330/339*	95.5c 279/292**c

DAY(S) = DAY(S) POSTPARTUM
a. Dosage occurred on day 7 of gestation through day 20 of lactation.
b. Excludes values for one pup from litter 8132, which had an accidental death.
c. Excludes values for pups that were sacrificed due to moribund sacrifice of dam.
d. Number of live pups on day 4 postpartum/number of liveborn pups on day 1 postpartum.
e. Number of live pups on day 21 postpartum/number of live pups on day 4 postpartum.
* Significantly different from the vehicle control group value (p≤0.05).
** Significantly different from the vehicle control group value (p≤0.01).

TABLE B12 (PAGE 3): LITTER OBSERVATIONS (NATURALLY DELIVERED PUPS) - SUMMARY - F1 GENERATION LITTERS

DOSAGE GROUP DOSAGE (MG/KG/DAY)a		I 0 (VEHICLE)	II 200	III 1000	IV 5000
DELIVERED LITTERS WITH ONE OR MORE LIVEBORN PUPS	N	23	24	25	25
LIVE LITTER SIZE AT WEIGHING					
DAY 1	MEAN±S.D.	13.2 ± 2.1	13.2 ± 2.0	13.7 ± 2.2	13.0 ± 2.5
DAY 4	MEAN±S.D.	13.1 ± 2.1	13.0 ± 1.8	13.6 ± 2.2	12.2 ± 2.8
DAY 7	MEAN±S.D.	13.1 ± 2.1	12.9 ± 2.1	13.5 ± 2.2	12.1 ± 2.6
DAY 14	MEAN±S.D.	13.0 ± 2.1	12.8 ± 2.2	13.2 ± 2.1	12.0 ± 2.6
DAY 21	MEAN±S.D.	13.0 ± 2.1	12.8 ± 2.2	13.0 ± 3.1 [24]b	11.6 ± 2.8 [24]c
PUP WEIGHT/LITTER (GRAMS)					
DAY 1	MEAN±S.D.	6.6 ± 0.5	6.4 ± 0.5	6.5 ± 0.3	6.0 ± 0.6**
DAY 4	MEAN±S.D.	9.2 ± 0.9	8.8 ± 1.1	8.9 ± 0.7	8.0 ± 1.2**
DAY 7	MEAN±S.D.	13.3 ± 1.5	12.4 ± 1.8	12.2 ± 1.5*	11.4 ± 1.8**
DAY 14	MEAN±S.D.	25.3 ± 4.0	24.8 ± 3.6	23.4 ± 3.7	21.5 ± 4.2**
DAY 21	MEAN±S.D.	39.4 ± 6.5	39.5 ± 5.6	37.0 ± 5.3 [24]b	35.1 ± 6.2* [24]c

DAY = DAY POSTPARTUM
[ ] = NUMBER OF VALUES AVERAGED
a. Dosage occurred on day 7 of gestation through day 20 of lactation.
b. Excludes values for litters 8153 and 8154; litters were combined on day 21 postpartum.
c. Excludes values for litter 8196; pups were sacrificed on day 20 postpartum due to moribund sacrifice of dam.
* Significantly different from the vehicle control group value (p≤0.05).
** Significantly different from the vehicle control group value (p≤0.01).

TABLE B14 (PAGE 1): NECROPSY OBSERVATIONS - SUMMARY - F1 GENERATION PUPS

MATERNAL DOSAGE GROUP MATERNAL DOSAGE (MG/KG/DAY)a		I 0(VEHICLE)	II 200	III 1000	IV 5000
LITTERS EVALUATED	N	23	24	25	25
TOTAL PUPS STILLBORN OR FOUND DEAD b,c	N	7	12	8	25
STILLBORN	N	4	6	3	5
FOUND DEAD	N	3	5	5	20
ACCIDENTAL DEATH	N	0	1	0	0
NO MILK IN STOMACH d	N(%)	1(33.3)	2(50.0)e	3(60.0)	8(44.4)f
PUPS SACRIFICED AND NECROPSIED ON DAY 20 OR 21 POSTPARTUM c					
LITTERS EVALUATED	N	23	24	25	25
PUPS EVALUATED	N	250	257	280	242
APPEARED NORMAL					
LITTER INCIDENCE	N(%)	23(100.0)	24(100.0)	25(100.0)	25(100.0)
PUP INCIDENCE	N(%)	250(100.0)	257(100.0)	280(100.0)	242(100.0)

a. Dosage occurred on day 7 of gestation through day 20 of lactation.
b. Restricted to pups in which complete necropsies were performed. Complete necropsies were not performed on pups in which autolysis or cannibalization precluded evaluation.
c. Refer to the individual pup clinical observations table (Table B25) for external clinical observations confirmed at necropsy.
d. Analysis restricted to pups found dead and necropsied.
e. Excludes values for one pup that did not have presence or absence of milk in stomach recorded.
f. Excludes values for two pups that did not have presence or absence of milk in stomach recorded.

F_1 generation results

One low dose male pup was found dead on Day 3 post-weaning with his death attributed to "failure to thrive" post-weaning. . No other male deaths were noted in the F1 generation during the post-weaning period. Two HDF_1 were found dead (sponsor's table below); only one death was considered drug-related by the sponsor.

TABLE C5 (PAGE 1): NECROPSY OBSERVATIONS - SUMMARY - F1 GENERATION FEMALE RATS

MATERNAL DOSAGE GROUP		I	II	III	IV
MATERNAL DOSAGE (MG/KG/DAY)		0 (VEHICLE)	200	1000	5000
RATS EXAMINED a	N	25	25	25	25
MORTALITY	N	1	0	0	2
FOUND DEAD	N	0	0	0	2b,c
MORIBUND SACRIFICED	N	1d	0	0	0
APPEARED NORMAL	N	25d	25	25	25b,c

a. Refer to the individual clinical observations table (Table C29) for external observations confirmed at necropsy.
b. Rat 9677 was found dead on day 57 postweaning.
c. Rat 9693 was found dead on day 5 postweaning.
d. Rat 9614 was moribund sacrificed on day 3 postweaning.

F_0 necropsy: No adverse lesions attributable to treatment with XP13512 were found at necropsy in any group. In males, testis and epididymis weights were similar among groups.

F_1 physical development: Development was comparable across dose groups. Dose-dependent decreased body weights were determined for all male dose groups and decreased body weight gains were noted at ≥1000 mg/kg/d (males only at 1000 mg/kg/d but both sexes at 5000 mg/kg/d). Body weights of females were not affected by treatment except at the high dose. Overall, feed consumption in treated animals was comparable to or higher than controls.
Unkempt coats were observed in the high dose pups (9 litters), presumably due to poor maternal caretaking. Pup weights were decreased on LD 7 in the 1000 and 5000 mg/kg/d groups and for the remainder of the lactation period for the high dose pups. Pup mortality was increased in the high dose group (3, 5, 5 and 20 pups for the respective dose groups).

Sponsor table and figure:

TABLE C3 (PAGE 1): TERMINAL BODY WEIGHT, TESTES AND EPIDIDYMIDES WEIGHTS AND RATIOS (%) OF TESTES AND EPIDIDYMIDES WEIGHTS TO TERMINAL BODY WEIGHT - SUMMARY - F1 GENERATION MALE RATS

MATERNAL DOSAGE GROUP		I	II	III	IV
MATERNAL DOSAGE (MG/KG/DAY)		0 (VEHICLE)	200	1000	5000
RATS TESTED	N	25	25	25	25
INCLUDED IN ANALYSES	N	25	24a	25	25
TERMINAL BODY WEIGHT	MEAN±S.D.	602.5 ± 45.2	566.7 ± 61.8*	555.0 ± 61.1**	527.4 ± 59.0**
EPIDIDYMIDES PAIRED	MEAN±S.D.	1.60 ± 0.22	1.51 ± 0.14	1.54 ± 0.19	1.48 ± 0.16
EPIDIDYMIDES PAIRED (%)	MEAN±S.D.	0.266 ± 0.036	0.268 ± 0.026	0.280 ± 0.036	0.282 ± 0.036
TESTES PAIRED	MEAN±S.D.	3.66 ± 0.34 [24]b	3.61 ± 0.35	3.57 ± 0.26	3.44 ± 0.28 [23]c
TESTES PAIRED (%)	MEAN±S.D.	0.610 ± 0.078 [24]b	0.643 ± 0.075	0.650 ± 0.082	0.656 ± 0.069 [23]c

ALL WEIGHTS WERE RECORDED IN GRAMS (G). RATIOS (%) = (ORGAN WEIGHT/TERMINAL BODY WEIGHT) X 100.
[ ] = NUMBER OF VALUES AVERAGED
a. Excludes values for rat 9542, which was found dead on day 3 postweaning.
b. Excludes values for rat 9518, which had abnormal organs (weight affected).
c. Excludes values for rats 9583 and 9591, which had the testes damaged (weight affected).
* Significantly different from the vehicle control group value (p≤0.05).
** Significantly different from the vehicle control group value (p≤0.01).

TABLE C9 (PAGE 1): BODY WEIGHT CHANGES - PRECOHABITATION - SUMMARY - F1 GENERATION FEMALE RATS

MATERNAL DOSAGE GROUP		I	II	III	IV
MATERNAL DOSAGE (MG/KG/DAY)		0 (VEHICLE)	200	1000	5000
RATS TESTED	N	25	25	25	25
INCLUDED IN ANALYSES	N	24a	25	25	24a
BODY WEIGHT CHANGE (G)					
DAYS 1 - 8	MEAN±S.D.	+33.1 ± 5.2	+34.6 ± 5.0	+32.5 ± 2.6	+30.4 ± 4.0*
DAYS 8 - 15	MEAN±S.D.	+42.9 ± 5.8	+42.5 ± 5.5	+42.4 ± 3.8	+38.2 ± 5.5**
DAYS 15 - 22	MEAN±S.D.	+40.5 ± 4.5	+38.7 ± 5.2	+40.7 ± 5.0	+39.3 ± 5.1
DAYS 22 - 29	MEAN±S.D.	+26.2 ± 6.1	+28.6 ± 5.2	+26.5 ± 5.0	+26.0 ± 5.8
DAYS 29 - 36	MEAN±S.D.	+23.4 ± 4.2	+22.6 ± 5.7	+24.2 ± 6.6	+22.8 ± 5.3
DAYS 36 - 43	MEAN±S.D.	+20.1 ± 5.7	+19.8 ± 7.0	+19.2 ± 5.5	+18.5 ± 7.4
DAYS 43 - 50	MEAN±S.D.	+17.9 ± 7.6	+17.5 ± 9.1	+18.0 ± 5.2	+15.7 ± 6.4
DAYS 50 - 57	MEAN±S.D.	+11.2 ± 5.6	+7.2 ± 13.0	+8.6 ± 6.1	+9.1 ± 5.8 [23]a
DAYS 57 - 64	MEAN±S.D.	+9.9 ± 5.3	+13.4 ± 12.0	+10.8 ± 5.5	+10.2 ± 6.5 [23]a
DAYS 64 - 71b	MEAN±S.D.	+11.2 ± 6.7	+11.4 ± 7.0	+8.0 ± 7.6	+9.3 ± 7.4 [23]a
DAYS 1 - 71b	MEAN±S.D.	+236.4 ± 20.8	+236.2 ± 29.2	+230.8 ± 21.3	+220.6 ± 27.6 [23]a
DAY 1 - PRECOHABITATION c	MEAN±S.D.	+243.0 ± 21.7	+242.6 ± 32.8	+235.1 ± 22.7	+225.7 ± 27.1 [23]a

DAY(S) = DAY(S) POSTWEANING
[ ] = NUMBER OF VALUES AVERAGED
a. Excludes values for rats that were found dead or moribund sacrificed.
b. Because body weight values were recorded at weekly intervals, based on each rat's day postweaning, day 71 postweaning was the last day on which the youngest rats had a body weight value recorded before cohabitation.
c. Precohabitation body weights were recorded on the day cohabitation began for the F1 generation rats; at that time these rats were 93 to 97 days of age.
* Significantly different from the vehicle control group value (p≤0.05).
** Significantly different from the vehicle control group value (p≤0.01).

TABLE C10 (PAGE 1): MATERNAL BODY WEIGHTS - GESTATION - SUMMARY - F1 GENERATION FEMALE RATS

MATERNAL DOSAGE GROUP		I	II	III	IV
MATERNAL DOSAGE (MG/KG/DAY)		0 (VEHICLE)	200	1000	5000
RATS TESTED	N	24a	24b	25	23a
PREGNANT	N	19	24	23	22
MATERNAL BODY WEIGHT (G)					
DAY 0	MEAN±S.D.	287.5 ± 26.4	280.2 ± 29.0	278.7 ± 20.3	260.4 ± 29.1**
DAY 7	MEAN±S.D.	323.3 ± 23.4	317.7 ± 31.5	310.1 ± 19.6	297.6 ± 29.5**
DAY 10	MEAN±S.D.	335.3 ± 24.7	329.8 ± 30.8	322.1 ± 20.5	307.1 ± 30.6**
DAY 14	MEAN±S.D.	357.3 ± 24.8	351.8 ± 34.2	344.0 ± 20.7	328.8 ± 31.2**
DAY 17	MEAN±S.D.	391.2 ± 26.3	382.1 ± 38.6	377.8 ± 21.0	360.0 ± 32.0**
DAY 21	MEAN±S.D.	466.3 ± 34.5	455.3 ± 48.2	449.6 ± 26.3	431.3 ± 39.6**

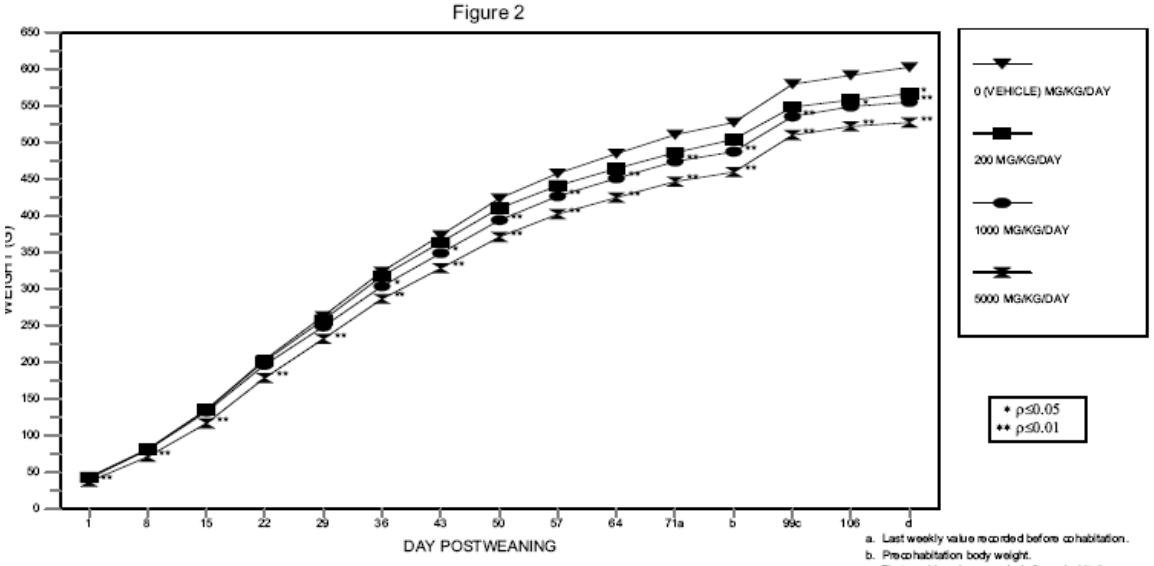
DAY = DAY OF GESTATION
a. Excludes values for rats that were found dead or moribund sacrificed before gestation.
b. Excludes values for rat 9645, which did not have a confirmed mating date.
** Significantly different from the vehicle control group value (p≤0.01).

TABLE C8 (PAGE 1): BODY WEIGHTS - PRECOHABITATION - SUMMARY - F1 GENERATION FEMALE RATS

MATERNAL DOSAGE GROUP		I	II	III	IV
MATERNAL DOSAGE (MG/KG/DAY)		0 (VEHICLE)	200	1000	5000
RATS TESTED	N	25	25	25	25
BODY WEIGHT (G)					
DAY 1	MEAN±S.D.	42.2 ± 5.6	42.3 ± 7.0	40.1 ± 4.6	36.1 ± 6.8**
DAY 8	MEAN±S.D.	75.5 ± 9.1 [24]a	76.9 ± 10.3	72.6 ± 6.0	66.8 ± 10.0** [24]a
DAY 15	MEAN±S.D.	118.4 ± 13.2 [24]a	119.4 ± 13.2	115.0 ± 8.1	105.1 ± 14.3** [24]a
DAY 22	MEAN±S.D.	158.9 ± 13.6 [24]a	158.1 ± 14.7	155.7 ± 9.8	144.4 ± 15.5** [24]a
DAY 29	MEAN±S.D.	185.1 ± 14.6 [24]a	186.7 ± 17.1	182.2 ± 12.5	170.4 ± 16.0** [24]a
DAY 36	MEAN±S.D.	208.5 ± 16.4 [24]a	209.3 ± 19.9	206.4 ± 13.3	193.2 ± 18.5** [24]a
DAY 43	MEAN±S.D.	228.6 ± 17.7 [24]a	229.1 ± 24.6	225.6 ± 16.8	211.7 ± 22.5** [24]a
DAY 50	MEAN±S.D.	246.5 ± 19.8 [24]a	246.6 ± 30.0	243.5 ± 18.8	227.4 ± 23.2** [24]a
DAY 57	MEAN±S.D.	257.7 ± 19.4 [24]a	253.8 ± 29.0	252.1 ± 19.8	237.7 ± 24.2** [23]a
DAY 64	MEAN±S.D.	267.6 ± 19.0 [24]a	267.2 ± 30.6	262.9 ± 20.8	248.0 ± 25.6** [23]a
DAY 71b	MEAN±S.D.	278.8 ± 21.4 [24]a	278.6 ± 31.8	270.8 ± 20.3	257.3 ± 29.8** [23]a
PRECOHABITATION c	MEAN±S.D.	285.4 ± 22.4 [24]a	284.9 ± 35.5	275.2 ± 21.6	262.4 ± 29.0** [23]a

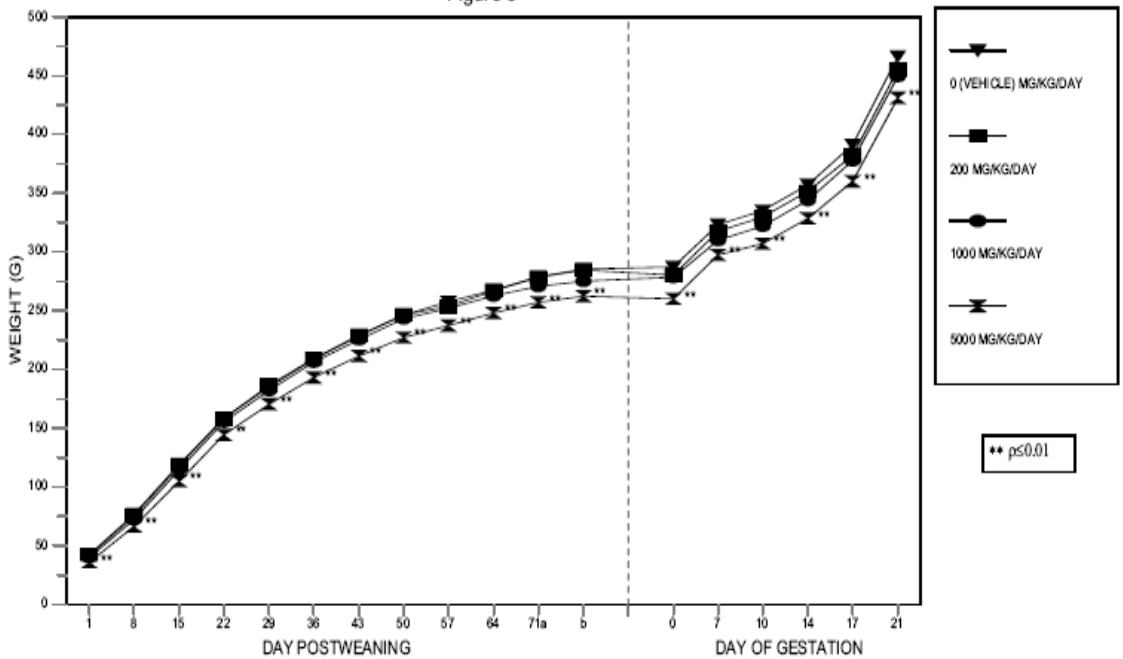
DAY = DAY POSTWEANING
[ ] = NUMBER OF VALUES AVERAGED
a. Excludes values for rats that were found dead or moribund sacrificed.
b. Because body weight values were recorded at weekly intervals, based on each rat's day postweaning, day 71 postweaning was the last day on which the youngest rats had a body weight value recorded before cohabitation.
c. Precohabitation body weights were recorded on the day cohabitation began for the F1 generation rats; at that time these rats were 93 to 97 days of age.
** Significantly different from the vehicle control group value (p≤0.01).

BODY WEIGHTS
F1 GENERATION MALE RATS
Figure 2



a. Last weekly value recorded before cohabitation.
b. Precohabitation body weight.
c. First weekly value recorded after cohabitation.
d. Terminal body weight.

BODY WEIGHTS
F1 GENERATION FEMALE RATS
Figure 3



a. Last weekly value recorded before cohabitation.
b. Precohabitation body weight.

Sponsor table:

TABLE C18 (PAGE 1): SEXUAL MATURATION - SUMMARY - F1 GENERATION RATS

MATERNAL DOSAGE GROUP		I	II	III	IV
MATERNAL DOSAGE (MG/KG/DAY)		0 (VEHICLE)	200	1000	5000
MALE RATS	N	25	24a	25	25
PREPUTIAL SEPARATION b	MEAN±S.D.	45.9 ± 2.3	46.2 ± 2.5	44.9 ± 2.0	47.1 ± 2.1
FEMALE RATS	N	24a	25	25	24a
VAGINAL PATENCY c	MEAN±S.D.	34.2 ± 1.6	33.2 ± 2.6	33.8 ± 1.5	34.8 ± 2.2

a. Excludes values for rats that were found dead or moribund sacrificed.
b. Average day postpartum that the prepuce was observed to be separated.
c. Average day postpartum that the vagina was observed to be patent.

F_1 behavioral evaluation: No adverse effects of treatment were found on learning, memory or response inhibition as tested in the passive avoidance or water maze testing.

F_1 reproduction: No adverse effects of treatment were found at any dose and mating and fertility were comparable across groups. C-section and litter parameters were not adversely affected at any dose.

Sponsor tables:

TABLE C21 (PAGE 1): MATING AND FERTILITY - SUMMARY - F1 GENERATION MALE RATS

MATERNAL DOSAGE GROUP		I	II	III	IV
MATERNAL DOSAGE (MG/KG/DAY)		0 (VEHICLE)	200	1000	5000
RATS IN COHABITATION	N	24a	24b	25	23a
DAYS IN COHABITATION d,e	MEAN±S.D.	3.1 ± 2.8	2.5 ± 1.3 [23]c	2.9 ± 1.9	2.5 ± 1.0
RATS THAT MATED e	N(%)	21(87.5)	24(100.0)	25(100.0)	22(95.6)
FERTILITY INDEX f,g	N/N	17/21	24/24	23/25	21/22
	(%)	(81.0)	(100.0)	(92.0)	(95.4)
RATS WITH CONFIRMED MATING DATES	N	21	23	25	22
MATED WITH FEMALE h					
DAYS 1-7	N(%)	19(90.5)	23(100.0)	24(96.0)	22(100.0)
DAYS 8-14	N(%)	2(9.5)	0(0.0)	1(4.0)	0(0.0)
RATS PREGNANT/RATS IN COHABITATION	N/N	17/24	24/24	23/25	21/23
	(%)	(70.8)	(100.0)**	(92.0)**	(91.3)**

a. Excludes values for rats that were not assigned to cohabitation due to no available female rat.
b. Excludes values for rat 9542, which was found dead on day 3 postweaning.
c. Excludes value for rat 9532, which did not have a confirmed mating date.
d. Restricted to rats with a confirmed mating date and rats that did not mate.
e. Includes only one mating for each male rat.
f. Number of pregnancies/number of rats that mated.
g. Includes only one pregnancy for each rat that impregnated more than one female rat.
h. Restricted to rats with a confirmed mating date.
** Significantly different from the vehicle control group value ($p \leq 0.01$).

TABLE C22 (PAGE 1): MATING AND FERTILITY - SUMMARY - F1 GENERATION FEMALE RATS

MATERNAL DOSAGE GROUP MATERNAL DOSAGE (MG/KG/DAY)		I 0 (VEHICLE)	II 200	III 1000	IV 5000
RATS IN COHABITATION	N	24a	25	25	23a
DAYS IN COHABITATION b	MEAN±S.D.	4.8 ± 5.2	2.6 ± 1.4 [24]c	2.9 ± 1.9	3.1 ± 3.2
RATS THAT MATED	N(%)	24(100.0)	25(100.0)	25(100.0)	23(100.0)
FERTILITY INDEX d	N/N (%)	19/24 (79.2)	25/25 (100.0)	23/25 (92.0)	22/23 (95.6)
RATS WITH CONFIRMED MATING DATES	N	24	24	25	23
MATED BY FIRST MALE e					
DAYS 1-7	N(%)	19(79.2)	24(100.0)**	24(96.0)**	22(95.6)**
DAYS 8-14	N(%)	2(8.3)	0(0.0)	1(4.0)	0(0.0)
MATED BY SECOND MALE e					
DAYS 15-21	N(%)	3(12.5)	0(0.0)	0(0.0)	1(4.3)
RATS PREGNANT/RATS IN COHABITATION	N/N (%)	19/24 (79.2)	25/25 (100.0)	23/25 (92.0)	22/23 (95.6)

a. Excludes values for rats that were found dead or moribund sacrificed.
b. Restricted to rats with a confirmed mating date and rats that did not mate.
c. Excludes value for rat 9645, which did not have a confirmed mating date.
d. Number of pregnancies/number of rats that mated.
e. Restricted to rats with a confirmed mating date.
** Significantly different from the vehicle control group value (p≤0.01).

TABLE C23 (PAGE 1): CAESAREAN-SECTIONING OBSERVATIONS - SUMMARY - F1 GENERATION FEMALE RATS

MATERNAL DOSAGE GROUP MATERNAL DOSAGE (MG/KG/DAY)		I 0 (VEHICLE)	II 200	III 1000	IV 5000
RATS TESTED	N	24a	25	25	23a
PREGNANT	N(%)	19(79.2)	25(100.0)	23(92.0)	22(91.7)
RATS PREGNANT AND CAESAREAN-SECTIONED ON DAY 21 OF GESTATION	N	19	25b	23	22
CORPORA LUTEA	MEAN±S.D.	17.1 ± 1.8	17.2 ± 4.0	16.6 ± 2.4	16.1 ± 1.7
IMPLANTATIONS	MEAN±S.D.	16.3 ± 1.7	16.3 ± 4.0	15.9 ± 2.1	15.2 ± 1.7
LITTER SIZES	MEAN±S.D.	15.6 ± 1.9	15.6 ± 4.1	15.4 ± 2.0	14.3 ± 2.4
LIVE FETUSES	N	297	389	355	315
	MEAN±S.D.	15.6 ± 1.9	15.6 ± 4.1	15.4 ± 2.0	14.3 ± 2.4
DEAD FETUSES	N	0	0	0	0
RESORPTIONS	MEAN±S.D.	0.6 ± 0.8	0.7 ± 1.0	0.4 ± 0.7	0.9 ± 1.9
EARLY RESORPTIONS	N	11	18	10	20
	MEAN±S.D.	0.6 ± 0.8	0.7 ± 1.0	0.4 ± 0.7	0.9 ± 1.9
LATE RESORPTIONS	N	1	0	0	0
	MEAN±S.D.	0.0 ± 0.2	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
DAMS WITH ANY RESORPTIONS	N(%)	9(47.4)	13(52.0)	8(34.8)	11(50.0)
DAMS WITH ALL CONCEPTUSES RESORBED	N(%)	0(0.0)	0(0.0)	0(0.0)	0(0.0)
DAMS WITH VIABLE FETUSES	N(%)	19(100.0)	25(100.0)	23(100.0)	22(100.0)
PLACENTAE APPEARED NORMAL	N(%)	19(100.0)	25(100.0)	23(100.0)	22(100.0)

a. Excludes values for rats that were found dead or moribund sacrificed before gestation.
b. Includes values for dam 9645, which did not have a confirmed mating date.

F_2 findings: No treatment-attributable necropsy findings were reported.

Sponsor tables:

TABLE C23 (PAGE 1): CAESAREAN-SECTIONING OBSERVATIONS - SUMMARY - F1 GENERATION FEMALE RATS

MATERNAL DOSAGE GROUP MATERNAL DOSAGE (MG/KG/DAY)		I 0 (VEHICLE)	II 200	III 1000	IV 5000
RATS TESTED	N	24a	25	25	23a
PREGNANT	N(%)	19(79.2)	25(100.0)	23(92.0)	22(91.7)
RATS PREGNANT AND CAESAREAN-SECTIONED ON DAY 21 OF GESTATION	N	19	25b	23	22
CORPORA LUTEA	MEANS.D.	17.1 ± 1.8	17.2 ± 4.0	16.6 ± 2.4	16.1 ± 1.7
IMPLANTATIONS	MEANS.D.	16.3 ± 1.7	16.3 ± 4.0	15.9 ± 2.1	15.2 ± 1.7
LITTER SIZES	MEANS.D.	15.6 ± 1.9	15.6 ± 4.1	15.4 ± 2.0	14.3 ± 2.4
LIVE FETUSES	N	297	389	355	315
	MEANS.D.	15.6 ± 1.9	15.6 ± 4.1	15.4 ± 2.0	14.3 ± 2.4
DEAD FETUSES	N	0	0	0	0
RESORPTIONS	MEANS.D.	0.6 ± 0.8	0.7 ± 1.0	0.4 ± 0.7	0.9 ± 1.9
EARLY RESORPTIONS	N	11	18	10	20
	MEANS.D.	0.6 ± 0.8	0.7 ± 1.0	0.4 ± 0.7	0.9 ± 1.9
LATE RESORPTIONS	N	1	0	0	0
	MEANS.D.	0.0 ± 0.2	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
DAMS WITH ANY RESORPTIONS	N(%)	9(47.4)	13(52.0)	8(34.8)	11(50.0)
DAMS WITH ALL CONCEPTUSES RESORBED	N(%)	0(0.0)	0(0.0)	0(0.0)	0(0.0)
DAMS WITH VIABLE FETUSES	N(%)	19(100.0)	25(100.0)	23(100.0)	22(100.0)
PLACENTAE APPEARED NORMAL	N(%)	19(100.0)	25(100.0)	23(100.0)	22(100.0)

a. Excludes values for rats that were found dead or moribund sacrificed before gestation.
b. Includes values for dam 9645, which did not have a confirmed mating date.

TABLE C24 (PAGE 1): LITTER OBSERVATIONS (CAESAREAN-DELIVERED FETUSES) - SUMMARY - F2 GENERATION LITTERS

MATERNAL DOSAGE GROUP MATERNAL DOSAGE (MG/KG/DAY)		I 0 (VEHICLE)	II 200	III 1000	IV 5000
LITTERS WITH ONE OR MORE LIVE FETUSES	N	19	25a	23	22
IMPLANTATIONS	MEANS.D.	16.3 ± 1.7	16.3 ± 4.0	15.9 ± 2.1	15.2 ± 1.7
LIVE FETUSES	N	297	389	355	315
	MEANS.D.	15.6 ± 1.9	15.6 ± 4.1	15.4 ± 2.0	14.3 ± 2.4
LIVE MALE FETUSES	N	157	192	168	158
% LIVE MALE FETUSES/LITTER	MEANS.D.	52.6 ± 13.2	51.4 ± 15.5	47.5 ± 10.8	50.3 ± 13.1
LIVE FETAL BODY WEIGHTS (GRAMS)/LITTER	MEANS.D.	5.21 ± 0.31	5.24 ± 0.32 [24]b	5.22 ± 0.27	5.27 ± 0.32
MALE FETUSES	MEANS.D.	5.30 ± 0.36	5.43 ± 0.35 [24]b	5.40 ± 0.30	5.41 ± 0.33
FEMALE FETUSES	MEANS.D.	5.11 ± 0.29	5.04 ± 0.31 [23]b,c	5.05 ± 0.26	5.12 ± 0.33
% RESORBED CONCEPTUSES/LITTER	MEANS.D.	3.9 ± 5.2	4.7 ± 7.3	2.6 ± 4.0	5.9 ± 12.6

[ ] = NUMBER OF VALUES AVERAGED
a. Includes values for litter 9645; the dam did not have a confirmed mating date.
b. Excludes values for litter 9645; the dam did not have a confirmed mating date.
c. Litter 9627 had no female fetuses.

2.6.6.7 Local tolerance

Three studies were conducted to assess local tolerance:
1) **LZ33462: Local Lymph Node Assay in the Mouse; Study #ED2008/00056/00 or 0102/0580**
2) **Primary Dermal Irritation/Corrosion in Rabbits; Study RD2008/00150/00XP063 or MB 05-13881.03**
3) **Primary Eye Irritation/Corrosion in Rabbits; Study RD2008/00151/00 or MB 05-13881.04**

LZ33462: Local Lymph Node Assay in the Mouse; Study #ED2008/00056/00 or 0102/0580; study initiation was 8/20/07.
This study was performed by (b) (4) in compliance with the SOPs for the laboratory and in compliance with OECD GLP standards.

A preliminary screening test was conducted at 50% w/w applied daily for 3 days. For the definitive test, 25 µL/dorsal ear surface of CBA/Ca mice (N=4/dose) were treated with a solution of 0% (vehicle), 10%, 25% or 50% w/w XP13512 (Lot 58 at 100.6% purity) in dimethyl formamide. Three days later, the mice were treated i.v. with ^{3}H-methyl thymidine (20µCi). All animals were euthanized 5 hrs later and the auricular lymph nodes were excised and pooled. Amounts of radioactivity were assessed via liquid scintillation counting. This laboratory considers a test positive if there is a 3x increase in radiolabel ("Stimulation Index") compared to concurrent controls.

Results: The "Stimulation Index" (mean radioactive incorporation/dose divided by the mean radioactive incorporation of the controls) was negative for all groups (0.98, 1.86 and 1.35 for the respective dose groups).
Sponsor table:

Table 2 Disintegrations per Minute, Disintegrations per Minute/Node and Stimulation Index

Concentration (% w/w) in dimethyl formamide	dpm	dpm/Node [a]	Stimulation Index [b]	Result
Vehicle	5936.74	742.09	na	na
10	5822.22	727.78	0.98	Negative
25	11036.27	1379.53	1.86	Negative
50	8019.12	1002.39	1.35	Negative

Under the conditions of this assay, XP13512 is a non-sensitizer.

Primary Dermal Irritation/Corrosion in Rabbits; Study RD2008/00150/00XP063 or MB 05-13881.03
This study was conducted by (b) (4) and was conducted in compliance with EPA GLP regulations and was initiated on 9/12/05.

Two male and 1 female NZW rabbits were treated dermally with 0.5 gms of XP13512 (Lot 9) (intact skin) and the sites were covered for 4 hrs. Evaluations were made 1 hr and 24, 48 and 72 hrs later.

Results: Neither erythema nor edema was seen at any time point. Thus, XP13512, under the conditions of this study, is not considered a dermal irritant.

Primary Eye Irritation/Corrosion in Rabbits; Study RD2008/00151/00 or MB 05-13881.04
This study was conducted by (b) (4) and was conducted in compliance with EPA GLP regulations and was initiated on 9/06/05.

Two male and 1 female NZW rabbits were treated into the conjunctival sac of one eye with 0.46 gms of XP13512 (Lot 9) and the sites were covered for 4 hrs. Evaluations (Draize methodology) were made 1 hr and 24, 48 and 72 hrs later.

Results: Fluorescein stain was retained in all treated eyes at 24 hrs post-dosing but no corneal opacities were appreciated. The conjunctiva was irritated in all animals by 1 hr post-dosing and cleared by Day 7. Thus, XP13512, under the conditions of this study, is a mild ocular irritant.
Sponsor table:

Table 1 **Ocular Findings and Systemic Observations**

An.#/Sex	Item	Tissue	Reading	Hour 1	Hour 24	Hour 48	Hour 72	Day 7
G7910/M	A	Cornea	Opacity	0	0	0	0	0
	B		Area	0	0	0	0	0
		1. Total=(AxB)x5		0	0	0	0	0
	C	Iris		0	0	0	0	0
		2. Total=Cx5		0	0	0	0	0
	D	Conjunctiva	Redness	2	2	1	1	0
	E		Chemosis	3	2	2	1	0
	F		Discharge	2 a	1	1	1	0
		3. Total=(D+E+F)x2		14	10	8	6	0
		Total=1+2+3		14	10	8	6	0
		Systemic Observations		A	A	A	A	A
		Sodium Fluorescein			1	0		

2.6.6.8 Special toxicology studies Not applicable

2.6.6.9 Discussion and Conclusions

See Summary of Nonclinical Studies

2.6.6.10 Tables and Figures

2.6.7 TOXICOLOGY TABULATED SUMMARY

Table 1. Toxicology — Overview — **Test Article:**

Type of Study	Species (strain)	No./ Group	Method of Administration	Salt Form	Dose (mg/kg/day) or Concentration	Duration of Dosing	GLP	Testing Facility	Report No. (Study No.)	L (
Single dose toxicity										
Single dose	Rat (Wistar)	3M/3F	Oral (gavage)	497	1000, 2000, 5000	Single	No	(b) (4)	RD2007/01519/00 (XP002)	r
Single dose	Rat (Wistar)	5M/5F	Oral (gavage)	512	2000, 5000	Single	Yes		RD2007/01697/00 (XP079)	r
Maximum tolerated dose	Monkey (cynomolgus)	1M/1F	Oral (gavage)	497	250, 500, 1000, 2000, 3500, 5000	Single	No		RD2007/01520/00 (XP003)	r
				512	1000, 2000					
Single dose	Monkey (cynomolgus)	1M/1F	Oral (gavage)	512	2000, 5000	Single	Yes		RD2007/01698/00 (XP080)	r
Repeat dose toxicity										
Dose range finding	Rat (Wistar)	5M/5F (6M/6F^{TK})	Oral (gavage)	512	500, 2000, 5000	7 days	No		RD2007/01519/00 (XP002)	r
Repeat dose toxicity	Rat (Wistar)	10M/10F (8M/8F^{TK})	Oral (gavage)	512	500, 2000, 5000	2 weeks	Yes		RD2007/01699/00 (XP007)	r
Repeat dose toxicity	Rat (Wistar)	10M/10F	Oral (gavage)	512[a]	500, 2000, 5000	2 weeks	Yes		RD2007/01525/00 (XP038)	r
Repeat dose toxicity	Rat (Wistar)	10M/10F	Oral (gavage)	512[a]	500, 2000, 5000	2 weeks	Yes		RD2007/01529/00 (XP049)	r
Repeat dose toxicity	Rat (Wistar)	15M/15F (15M/15F^{TK})	Oral (gavage)	512	500, 2000, 5000	13 weeks	Yes		RD2007/01523/00 (XP025)	r

								(b) (4)	
Repeat dose toxicity (continued)									
Repeat dose toxicity	Rat (Wistar)	25M/25F[b] (12M/12F[TK])	Oral (gavage)	512	500, 2000, 5000	26 weeks	Yes		RD2007/01526/00 (XP046)
Dose range finding	Monkey (cynomolgus)	2M/2F	Oral (gavage)	512	500, 2000	7 days	No		RD2007/01520/00 (XP003)
Repeat dose toxicity	Monkey (cynomolgus)	3M/3F	Oral (gavage)	512	250, 750, 2000	2 weeks	Yes		RD2007/01521/00 (XP008)
Repeat dose toxicity	Monkey (cynomolgus)	4M/4F	Oral (gavage)	512	500, 1000, 2000	13 weeks	Yes		RD2007/01524/00 (XP026)
Repeat dose toxicity	Monkey (cynomolgus)	6M/6F[c]	Oral (gavage)	512	250, 1000, 2000	39 weeks	Yes		RD2007/01528/00 (XP047)
Genotoxicity									
Ames test	*S. typhimurium* *E. coli*	NA	In vitro	512	33.3 to 5000 µg/plate	NA	Yes		RD2007/01492/00 (XP014)
Ames test	*S. typhimurium* *E. coli*	NA	In vitro	512[a]	33.3 to 5000 µg/plate	NA	Yes		RD2007/01491/00 (XP029)
Ames test	*S. typhimurium* *E. coli*	NA	In vitro	512[a]	33.3 to 5000 µg/plate	NA	Yes		RD2007/01490/00 (XP048)
Chromosomal aberration assay	Human lymphocytes	NA	In vitro	512	24 to 3500 µg/mL	NA	Yes		RD2007/01489/00 (XP013)
Micronucleus assay	Rat (Wistar)	6M 12M	Oral (gavage)	512	500, 1000 2000	Single	Yes		RD2007/01488/00 (XP015)
Genotoxicity (continued)									
Unscheduled DNA synthesis	Rat (SD)	4M	Oral (gavage)	512	500, 1000, 2000	Single	Yes		RD2008/00149/00 (XP062)
Carcinogenicity									
Dose range finding	Mouse (B6C3F1)	10M/10F (30M/30F[TK])	Oral (gavage)	512	500, 2000, 5000	7 days	No		RD2007/01522/00 (XP023)
Pre-oncogenicity	Mouse (B6C3F1)	15M/15F (38M/38F[TK])	Oral (gavage)	512	500, 2000, 5000	13 weeks	Yes		RD2008/00324/00 (XP024)
Carcinogenicity	Mouse (B6C3F1)	60M/60F	Oral (gavage)	512	500, 2000, 5000	2 years	Yes		RD2008/00346/00 (XP050)
Carcinogenicity	Rat (Wistar)	60M/60F	Oral (gavage)	512	500, 2000, 5000	2 years	Yes		RD2008/00347/00 (XP051)
Reproductive and Developmental Toxicity									
Fertility	Rat (SD)	25M 25F	Oral (gavage)	512	200, 1000, 5000	4 weeks prior to cohabitation through cohabitation 2 weeks prior to cohabitation through Day 7 pc	Yes		RD2008/00147/00 (XP035)
EFD (DRF)	Rat (SD)	8F	Oral (gavage)	512	200, 500, 1500, 5000	Days 7 to 17 pc	Yes		RD2007/01534/00 (XP031)

Reproductive and Developmental Toxicity (continued)

								(b) (4)		
EFD	Rat (SD)	25F (6F[TK])	Oral (gavage)	512	200, 1000, 5000	Days 7 to 17 pc	Yes		RD2008/00144/00 (XP032)	
EFD (DRF)	Rabbit (NZW)	5F (3F[TK])	Oral (stomach tube)	512	200, 500, 1500, 5000	Days 7 to 19 pc	Yes		RD2008/00145/00 (XP033)	
EFD	Rabbit (NZW)	20F (3F[TK])	Oral (stomach tube)	512	200, 500, 2500	Days 7 to 19 pc	Yes		RD2008/00146/00 (XP034)	
Peri- & post-natal development	Rat (SD)	25F	Oral (gavage)	512	200, 1000, 5000	Day 7 pc to Day 20 pp	Yes		RD2008/00148/00 (XP036)	
Local Tolerance										
Dermal irritancy	Rabbit (NZW)	2M/1F	Topical	512	0.5 g	Single	Yes		RD2008/00150/00 (XP063)	
Ocular irritancy	Rabbit (NZW)	2M/1F	Placed in the conjunctival sac	512	46 mg	Single	Yes		RD2008/00151/00 (XP064)	
Local lymph node assay	Mouse (CBA/Ca)	4F	Topical	512	10%, 25%, 50%	Single	Yes		ED2008/00056/00 (0102/0580)	
Other Toxicity Studies										
Stability	Human whole blood	NA	In vitro	512	1500, 4500 µM	NA	No		RD2008/00754/00 (XP020)	
Genotoxicity (impurities)	NA	NA	In vitro (Ames)	I1	0.5 to 1500 µg/plate	NA	Yes		ED2008/00053/00 (0102/0556)	
Other Toxicity Studies (continued)										
Genotoxicity (impurities)	NA	NA	In vitro (Ames)	I1	50 to 5000 µg/plate	NA	No		WD2008/00530/00 (Ames-182)	m4.2.3.7.6
Genotoxicity (impurities)	NA	NA	In vitro (Ames)	I2	50 to 5000 µg/plate	NA	Yes		ED2008/00070/00 (0102/0567)	m4.2.3.7.6
Genotoxicity (impurities)	NA	NA	In vitro (Ames)	I3	1.5 to 5000 µg/plate	NA	Yes		CD2008/00969/00 (0102/0618)	m4.2.3.7.6
Chemical stability	NA	NA	In vitro	512, I4	5 µM	NA	No		RD2008/01160/00 (XP098)	m4.2.3.7.6
Metabolic stability	Human liver & intestinal S9									

Key:
SD = Sprague Dawley.
NZW = New Zealand white.
497 (b) (4)
512
I1 =
I2 =
I3 =
I4 =

M = Male
F = Female.
NA = Not applicable.
pc = post coitum.
pp = post partum.
EFD = Embryofetal development.
DRF = Dose range finding.

Testing Facility: (b) (4)

TK = Additional animals included for toxicokinetic investigations only.

a. Used batch containing potential impurities for qualification purposes.
b. Three to five animals/sex/group were maintained to evaluate a one month recovery period.
c. Two animals/sex/group were euthanized at 26 weeks for interim necropsy. An additional 2 animals/sex/group were included in the control and 2000 mg/kg/day group to evaluate a one month recovery period.

Table 2. Toxicokinetics — Overview of Toxicokinetics Studies — Test Article:

Type of Study	Test System	Method of Administration	Doses (mg/kg)	GLP Compliance	Report (Study Number)	L (
Maximum tolerated dose	Monkey (cynomolgus)	Oral (gavage)	250, 500, 1000, 2000, 3500, 5000[b]	No	RD2007/01520/00 (XP003)	r
			1000, 2000			
Single dose	Monkey (cynomolgus)	Oral (gavage)	2000, 5000	Yes	RD2007/01698/00 (XP080)	r
Dose range finding (7 days)	Rat (Wistar)	Oral (gavage)	500, 2000, 5000	No	RD2007/01519/00 (XP002)	r
Repeat dose toxicity (2 weeks)	Rat (Wistar)	Oral (gavage)	500, 2000, 5000	Yes	RD2007/01699/00 (XP007)	r
Repeat dose toxicity (2 weeks)	Rat (Wistar)	Oral (gavage)	500, 2000, 5000[c]	Yes	RD2007/01525/00 (XP038)	r
Repeat dose toxicity (2 weeks)	Rat (Wistar)	Oral (gavage)	500, 2000, 5000[c]	Yes	RD2007/01529/00 (XP049)	r
Repeat dose toxicity (13 weeks)	Rat (Wistar)	Oral (gavage)	500, 2000, 5000	Yes	RD2007/01523/00 (XP025)	r
Repeat dose toxicity (26 weeks)	Rat (Wistar)	Oral (gavage)	500, 2000, 5000	Yes	RD2007/01526/00 (XP046)	r
Dose range finding (7 days)	Monkey (cynomolgus)	Oral (gavage)	500, 2000	No	RD2007/01520/00 (XP003)	r
Repeat dose toxicity (2 weeks)	Monkey (cynomolgus)	Oral (gavage)	250, 750, 2000	Yes	RD2007/01521/00 (XP008)	r
Repeat dose toxicity (13 weeks)	Monkey (cynomolgus)	Oral (gavage)	500, 1000, 2000	Yes	RD2007/01524/00 (XP026)	r
Repeat dose toxicity (39 weeks)	Monkey (cynomolgus)	Oral (gavage)	250, 1000, 2000	Yes	RD2007/01528/00 (XP047)	
Carcinogenicity (7 day dose range finding)	Mouse (B3C3F1)	Oral (gavage)	500, 2000, 5000	No	RD2007/01522/00 (XP023)	I
Pre-oncogenicity	Mouse (B3C3F1)	Oral (gavage)	500, 2000, 5000	Yes	RD2008/00324/00 (XP024)	I
Embryofetal development	Rat (Sprague Dawley)	Oral (gavage)	200, 1000, 5000	Yes	RD2008/00144/00 (XP032)	I
Embryofetal development (dose range finding)	Rabbit (New Zealand white)	Oral (stomach tube)	200, 500, 1500, 5000	Yes	RD2008/00145/00 (XP033)	I
Embryofetal development	Rabbit (New Zealand white)	Oral (stomach tube)	200, 500, 2500	Yes	RD2008/00146/00 (XP034)	I

OVERALL CONCLUSIONS AND RECOMMENDATIONS

See Executive Summary

Recommendations: The sponsor has conducted an exhaustive battery of tests for this pro-drug of gabapentin. In most instances, the toxicologic profile is identical or essentially similar to that found for gabapentin. The human risk assessment for the product needs to take into consideration the potential renal ramifications and pancreatic toxicity and tumors noted in the rat studies.

Appendix/attachments

Executive CAC
Date of Meeting: August 4, 2009

Committee: David Jacobson-Kram, Ph.D., OND IO, Chair
Paul Brown, Ph.D., OND IO, Member
Todd Bourcier, Ph.D., DMEP, Alternate Member
Lois Freed, Ph.D., DNP Supervisor
Terry Peters, D.V.M., DNP Presenting Reviewer

Author of Draft: Terry Peters

The following information reflects a brief summary of the Committee discussion and its recommendations.

NDA #22399
Drug Name: (b) (4)™ (proposed name)
Sponsor: GlaxoSmithKline

Background: (b) (4)™ is a novel prodrug of gabapentin intended for treatment of moderate to severe primary Restless Legs Syndrome.

The test article was not genotoxic in the in vitro Ames test, the in vivo micronucleus or the in vivo/in vitro UDS assay, but it was positive in the in vitro chromosomal aberration assay in human lymphocytes. The positive finding was attributed to in vitro release of acetaldehyde during the (b) (4)

The protocols for the lifetime carcinogenicity studies in mouse and rat were reviewed by the ECAC on 5/3/05 and the Committee concurred with the sponsor's proposed doses for both studies.

Rat Carcinogenicity Study
Wistar rats were treated for up to 104 weeks with 0, 500, 2000 or 5000 mg/kg/d of XP13512 in Tween 80 and methylcellulose by oral gavage. The 2000 and 5000 mg/kg/d males were terminated early (Weeks 97 and 90, respectively) due to exacerbation of chronic progressive nephropathy. Females were not similarly affected. There was an increased incidence of pancreatic acinar cell hyperplasia, adenomas, and adenomas + carcinomas in both sexes at 5000 mg/kg/d and in males at 2000 mg/kg/d. The study appears to have been appropriately conducted and the mid and high doses elicited toxicity as well as tumors. Thus XP13512 is considered a carcinogen in rats under the conditions of this study.

Combined Pancreatic Lesions in Rats Treated with XP13512 for Up to 104 Weeks

	Males				Females			
Dose (mg/kg/d)	**0**	**500**	**2000**	**5000**	**0**	**500**	**2000**	**5000**
Hyperplasia, acinar; min-mild	11	1	8	0	11	3	17	10
Mod-severe	3	0	2	1	3	1	3	4
Acinar adenoma	2	4	4	8	0	0	0	3
Acinar carcinoma	0	0	1	1	0	0	0	1

Mouse Carcinogenicity Study

B6C3F$_1$/Crl mice were treated with XP13512 in Tween 80 and methylcellulose by oral gavage at 0, 500, 2000 or 5000 mg/kg/d for up to 104 weeks. Decreased survival was found in mid (83% compared to controls) and high dose (83% compared to controls) males; however, a sufficient number of animals survived to scheduled termination to consider the study valid. Body weight was increased in the high dose males and females, compared to controls. No other significant adverse effects of treatment were found other than a minimal to mild exacerbation of age-related axonal/ myelin degeneration of the sciatic nerve found in mid dose females and both sexes at the high dose. No drug-related increases of any tumor type were found in this study.

Executive CAC Recommendations and Conclusions:

Rat:

- The Committee concluded that the study was adequate.
- The Committee found that the study was positive for carcinogenicity, noting increases in pancreatic acinar cell hyperplasia, adenomas, and adenomas + carcinomas in males at 2000 and 5000 mg/kg/d and in females at 5000 mg/kg/d.
- A survival adjusted statistical analysis of tumor incidences is pending.

Mouse:

- The Committee concluded that the study was adequate and negative for carcinogenicity.
- A survival adjusted statistical analysis of tumor incidences is pending.

David Jacobson-Kram, Ph.D.
Chair, Executive CAC

cc:\
/Division File, DNP
Freed/Team leader, DNP
Peters/Reviewer, DNP
Connor/CSO/PM, DNP
/ASeifried, OND IO

Executive CAC
May 3, 2005

Committee: David Jacobson-Kram, Ph.D., HFD-024, Chair
Joseph Contrera, Ph.D., HFD-901, Member
Abby Jacobs, Ph.D., HFD-024, Member
Adebayo Laniyonu, Ph.D., HFD-160, Alternate Member
Lois Freed, Ph.D., HFD-120, Supervisory Pharmacologist
Ed Fisher, Ph.D., HFD-120, Presenting Reviewer

Author of Minutes: Ed Fisher

The following information reflects a brief summary of the Committee discussion and its recommendations. Detailed study information can be found in the individual review.

The committee did not address the sponsor's proposed statistical evaluation for the 2-yr carcinogenicity bioassays, as this does not affect the sponsor's ability to initiate the bioassays. The sponsor may seek guidance on the statistical evaluation of bioassay results from agency staff separately. Data files should be submitted electronically following section E of the 'Guidance for Industry, Providing Regulatory Submission in Electronic Format, New Drug Application.'

IND #: 71,352
Drug Name: XP13512
Sponsor: XenoPort

Background:

XP13512 undergoes enzymatic hydrolysis to form gabapentin, acetaldehyde, isobutyric acid, and carbon dioxide. Gabapentin is excreted essentially unchanged in humans as well as B6C3F1 mice and Wistar rats. XP13512 was negative in the Ames and rat micronucleus tests, but positive in human lymphocytes (attributed to the acetaldehyde byproduct). Gabapentin was negative in the genotoxicity battery.

Mouse carcinogenicity study protocol and dose selection

Doses proposed for the carcinogenicity study in B6C3F1 mice were based on the results of a 13-week mouse (B6C3F1; 15/sex/group) toxicity study with doses of 0, 500, 2000, and 5000 mg/kg/day (oral gavage; 20 mL/kg). No treatment-related effects were observed on any of the parameters evaluated during the study. Body weights were generally higher in both sexes at the MD and HD compared to C, but this was not considered toxicologically significant.

The proposed HD of 5000 mg/kg XP13512 was considered a maximum feasible dose based on the combination of the maximum technically feasible daily oral gavage volume of 20 mL/kg/day and the maximum technically feasible concentration of the dosing formulation of 250 mg/mL (20 mL/kg/day X 250 mg/mL = 5000 mg/kg/day). At this concentration, the dosing formulation was described as a thick, viscous suspension. This dose produces 9-fold the maximum anticipated clinical exposure on an AUC basis.

Rat carcinogenicity study protocol and dose selection

Doses proposed for the carcinogenicity study in Wistar rat were based on the results of a 13-week rat (Wistar; 15/sex/group) toxicity study with doses of 0, 500, 2000, and 5000 mg/kg/day (oral gavage; 20 mL/kg). No effects on survival occurred. A transient decrease in activity was noted in all treated groups of both sexes, accompanied by salivation at the MD and HD. Mean body weight and food consumption values were higher than C at all doses in both sexes. These

findings were not considered toxicologically important. No treatment-related ophthalmology, hematology, or urinalysis changes were observed. Cholesterol was moderately increased in HD females. No treatment-related macroscopic changes were seen. Microscopically, treatment-related changes consisted of centrilobular hepatocellular hypertrophy at the HD in both sexes (liver weights were moderately increased in both sexes at the MD and HD), hyaline droplet accumulation in the tubular epithelium in males at all doses, and related chronic progressive nephropathy in MD and HD males (kidney weights were moderately increased in MD males and in both sexes at the HD). None of these findings was considered dose-limiting.

The proposed HD of 5000 mg/kg XP13512 was considered a maximum feasible dose based on the combination of the maximum technically feasible daily oral gavage volume of 20 mL/kg/day and the maximum technically feasible concentration of the dosing formulation of 250 mg/mL (20 mL/kg/day X 250 mg/mL = 5000 mg/kg/day). At this concentration, the dosing formulation was described as a thick, viscous suspension. This dose produces 30-fold the maximum anticipated clinical exposure on an AUC basis.

Executive CAC recommendations and conclusions:

The Committee concurred with the sponsor's proposed doses of 0, 500, 2000, and 5000 mg/kg/day by oral gavage in males and females for both the mouse and rat carcinogenicity studies, based on the information provided by the sponsor indicating that the high dose is a maximum feasible dose, due to viscosity at the maximum volume and highest concentration. Although the Committee noted that the proposed dosing volume (20 mL/kg) may be larger than routinely used in a 2-year study, the data from the 13-week studies indicated that the 20 mL/kg volume was well-tolerated in both species.

The sponsor should consult the Division if dose adjustment or early termination is considered for either study.

David Jacobson-Kram, Ph.D.
Chair, Executive CAC

cc:\
/Division File, HFD 120
/LFreed, HFD-120
/EFisher, HFD-120
/TWheelous, HFD-120
/ASeifried, HFD-024

Application Type/Number	Submission Type/Number	Submitter Name	Product Name
NDA-22399	ORIG-1	GLAXO GROUP LTD DBA GLAXOSMITHKLINE	SOLZIRA

This is a representation of an electronic record that was signed electronically and this page is the manifestation of the electronic signature.

/s/

TERRY S PETERS
02/01/2010

LOIS M FREED
02/01/2010
Please see memo for comments.