Table IVC.2: Body weights in 2-year rat carcinogenicity study of GP 47779 (sponsor's table)

Mean body weight change during the dosing period

Group dose (mg/kg)	Males			Females		
	Body weight (g)		Percent	Body weight (g)		Percent
	baseline	week 105	change[a]	baseline	week 105	change[a]
1 (0)	196.3	714.0	258.8 (-)	153.3	481.2	221.5 (-)
2 (75)	191.5	796.7	323.0 (16.9)	152.5	508.6	244.8 (8.6)
3 (250)	193.4	768.8	311.2 (11.1)	153.5	421.0**	178.6** (-18.4)
4 (600)	191.0	604.0**	220.0** (-20.2)	152.6	308.9**	101.6** (-52.3)

a = Values in parentheses represent:

Percent gain relative to control = $\frac{\text{weight gain of group - weight gain of control} \times 100}{\text{weight gain of control}}$

**Statistically significant relative to the control group at $p < 0.01$.

d. Ophthalmologic Examinations (performed predose and at 51 and 103 weeks)

No T-R ocular changes were observed.

e. Hematology (performed at 104 weeks on 10/sex/grp)

Erythrocyte parameters tended to be increased in treated males (HGB SS at HD) and WBCs decreased in treated females.

f. Gross Pathology (complete necropsy performed on all rats)

Numbers of animals with grossly observable hepatic nodules (reported as tissue masses) and lesions were increased in all treatment groups compared to C **(Table IVC.3)**.

Table IVC.3 Summary of gross liver changes

Dose	0		75		250		600	
Sex	M	F	M	F	M	F	M	F
N	60	60	60	60	60	60	60	60
With tissue mass(es)	2	1	7	7	9	17	17	32
With lesion(s)	26	21	32	28	38	42	40	42

g. Microscopic Pathology (complete microscopic examinations were performed on all animals)

i. Non-proliferative

Treatment increased the incidences and/or severity of renal lesions at all doses (**Table IVC.4**). These included typical chronic nephropathy as well as less common findings such as thrombosis of large caliber hilar veins in males and (golden brown granular) pigmentation of the renal tubular epithelium in females. Not included in the sponsor's summary table is an increased incidence of hydronephrosis in females (3/60, 5/60, 7/60, 7/60).

Incidences of various non-proliferative liver alterations were also increased in treated rats at all dose levels (**Table IVC.5**). Not included in the summary table is an increased incidence of liver thrombosis in females (0/60, 2/60, 2/60, 4/60).

Other findings included increased incidences of cerebral mineralization in MD and HD males (2/60, 1/60, 5/60, 6/60) and HD females (0/60, 2/60, 0/60, 5/60); foamy macrophage accumulation in the lung in HD males (8/60, 10/60, 6/60, 18/60) and females (7/60, 7/60, 12/60, 20/60); and thymic atrophy (12/57, 14/57, 24/55, 27/57), thymic cysts (7/57, 6/57, 11/55, 19/57), and splenic pigmented macrophages (20/60, 22/60, 27/60, 32/60) in treated females.

Table IVC.4: **Summary of compound-related microscopic kidney changes**

	Males				Females			
Group:	1	2	3	4	1	2	3	4
Dose (mg/kg):	0	75	250	600	0	75	250	600
Number examined:	60	60	60	60	60	60	60	60
Chronic progressive nephropathy,								
minimal	20	13	17	11	15	13	14	11
mild	14	27	17	22	5	17	17	20
moderate	7	11	4	8	2	6	13	12
severe	10	7	21	18	3	2	2	10
All severities	51	58	59	59	25	38	46	53
Thrombus	0	1	2	5	0	0	0	1
Pigmentation, tubular, epithelial	1	2	1	0	1	6	3	21

Table IVC.5

Summary of compound-related microscopic liver changes

	Males				Females			
Group:	1	2	3	4	1	2	3	4
Dose (mg/kg)	0	75	250	600	0	75	250	600
Number examined:	60	60	60	60	60	60	60	60
Proliferative lesions								
Foci of cellular alteration, acidophilic	17	34	35	51	6	21	39	40
Hyperplasia, regenerative	0	0	1	0	0	0	3	5
Hepatocellular adenoma	2	5	5	19	2	3	15	28
Hepatocellular carcinoma	2	5	3	6	0	0	6	11
Hepatocellular adenoma and/or carcinoma	4	9	8	24	2	3	20	35
Biliary hyperplasia	24	30	32	32	22	25	36	37
Non-proliferative lesions								
Hypertrophy, hepatocellular, centrilobular	0	30	59	55	0	40	54	53
Angiectasis	19	21	22	24	14	21	30	26
Cyst, biliary, multilocular	0	1	0	2	2	3	8	14
Degeneration, cystic	22	27	43	44	1	7	21	25
Fibrosis, periportal	17	18	22	23	5	10	17	16
Necrosis, hemorrhagic	0	2	2	2	0	2	3	12
Mineralization	1	1	0	1	0	0	0	4
Pigment, hepatocellular	2	0	1	3	0	4	11	25
Pigmented macrophages	12	7	23	18	10	14	25	37
Vacuolation, hepatocellular	19	21	31	31	10	16	17	21

ii. Proliferative

Treatment with GP 47779 was associated with increased incidences of liver tumors and hyperplasia, interstitial cell tumors of the testis, granular cell aggregates or tumors of the vagina and cervix, and thyroid follicular cell hypertrophy/hyperplasia. The spectrum of proliferative liver changes, which were increased in incidence and/or severity at all doses, included foci of cellular alteration, regenerative hyperplasia, and neoplasia (**Table IVC.5**). In addition to an increase in interstitial cell tumors in MD and HD males, focal hyperplasia of interstitial cells was increased somewhat at all doses (**Table IVC.6**). Proliferative lesions of the female reproductive tract were also increased at all doses (**Table IVC.7**). Thyroid follicular hyperplasia/hypertrophy was found in 1/60, 3/60, 2/60, and 6/60 males and 0/60, 1/60, 2/60, and 8/60 females in the C, LD, MD, and HD groups, respectively.

Table IVC.6 Summary of microscopic testicular proliferative changes

Dose	0	75	250	600
N	60	60	60	60
Hyperplasia, interstitial cell, focal	1	3	2	3
Interstitial cell tumor, benign	1	1	**7**	**6**

Table IVC.7 Summary of compound-related microscopic changes of the cervix/vagina

Dose	0	75	250	600
N	60	60	60	60
Granular cell aggregate(s), cervix or vagina	1	2	2	1
Granular cell tumor, benign, cervix or vagina	0	**4**	**4**	**6**
Granular cell tumor, malignant, vagina	0	0	1	1
Combined granular cell aggregate(s), benign or malignant tumor	1	**6**	**7**	**8**

h. Toxicokinetics

Mean plasma levels (n=5/sex/group) of GP 47779 and CGP 10000 measured at 2 hr after dosing during weeks 1 and 52 are shown in **Table IVC.8**. GP 47779 levels at this single time point were consistently higher in females and generally increased over the course of the study in both sexes (except in HD females).

Table IVC.8. Plasma levels (mean±SD) of GP 47779 and CGP 10000 2 hours after oral administration of GP 47779 to rats in a 2-year carcinogenicity study

		Plasma levels (ng/ml)			
		Week 1		Week 52	
Dose (mg/kg)	Sex	GP 47779	CGP 10000	GP 47779	CGP 10000
75	M	1266 ± 770	BQL	3007 ± 1115	BQL
	F	3174 ± 1527	BQL	4083 ± 3371	2453 ± 779
250	M	4109 ± 1962	2535 ± 675	5561 ± 3096	2419 ± 876
	F	6293 ± 3211	2149 ± 1045	8631 ± 4625	2877 ± 1009
600	M	5534 ± 1619	3118 ± 358	9883 ± 2195	3648 ± 982
	F	20842 ± 24734	4196 ± 1855	15655 ± 4519	7038 ± 2233

V. GENETIC TOXICITY

A. AMES TEST WITH SYNTHESIS 2 GP 47680 (Tox. Ref. 3-14, Test no. 936205, conducted by [redacted] 1994, GLP, Vol. 1.67)

When GP 47680 (Batch no. 800189; dissolved in DMSO) was evaluated in tester strains TA1537, TA1535, TA100, TA98, and WP2 uvrA at concentrations ranging from 39 to 5000 ug/plate (concentrations ≥1250 ug/plate inhibited bacterial growth) with and without S-9 (incubation at 37C for 48 hr), small (up to ~2X C) but concentration-dependent increases in incidences of histidine-prototrophic mutants were observed at all concentrations up to those that inhibited growth in strain TA 100 without metabolic activation (**Table VA**). This effect was confirmed in additional experiments using different batches (800192, A 45/114.2) and a new delivery of batch 800189 (i.e., seen in original plus 3 confirmatory experiments). Appropriate control responses were observed.

Table VA

: MUTAGENICITY TEST
Experiment without metabolic activation

Test number : 936205
Experiment : 3rd confirmatory
Test substance : GP 47 680
Batch : 800189 old
Strain : TA 100

Treatment	Colony counts			Mean
Negative control	103	132	115	116.67
GP 47 680:				
39.06 µg/plate	177	175	161	171.00
78.13 µg/plate	168	187	177	177.33
156.25 µg/plate	209	220	248	225.67
312.50 µg/plate	242	256	217	238.33
625.00 µg/plate	230	247	281	252.67
1250.00 µg/plate	187	151	161	166.33
2500.00 µg/plate	4	16	14	11.33
5000.00 µg/plate	0	0	0	0.00
Sodium azide 5.00 ug/plate	1141	1276	1371	1262.67

Experiment : 3rd confirmatory
Test substance : GP 47 680
Batch : 800189 new
Strain : TA 100

Treatment	Colony counts			Mean
Negative control	105	103	114	107.33
GP 47 680:				
39.06 µg/plate	120	108	156	128.00
78.13 µg/plate	189	170	180	179.67
156.25 µg/plate	172	174	230	192.00
312.50 µg/plate	231	210	224	221.67
625.00 µg/plate	221	194	228	214.33
1250.00 µg/plate	163	165	110	146.00
2500.00 µg/plate	21	12	17	16.67
5000.00 µg/plate	0	0	0	0.00
Sodium azide 5.00 ug/plate	1191	1398	1350	1313.00

Table VA cont.

: MUTAGENICITY TEST
Experiment without metabolic activation

Test number : 936205
Experiment : 3rd confirmatory
Test substance : GP 47 680
Batch : A 45/114.2
Strain : TA 100

Treatment	Colony counts			Mean
Negative control	124	121	116	120.33
GP 47 680:				
39.06 µg/plate	149	162	152	154.33
78.13 µg/plate	157	163	139	153.00
156.25 µg/plate	204	200	209	204.33
312.50 µg/plate	253	241	225	239.67
625.00 µg/plate	266	246	268	260.00
1250.00 µg/plate	177	218	176	190.33
2500.00 µg/plate	64	27	69	53.33
5000.00 µg/plate	0	0	0	0.00
Sodium azide 5.00 ug/plate	1253	1364	1359	1325.33

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B. REPEAT AMES TEST WITH SYNTHESIS 2 GP 47680 (Tox. Ref. 3-15, Study no. 971623, conducted by Novartis in 1998, GLP, Vol 1.67)

GP 47680 (batch no. 400697, in DMSO) was evaluated in strains TA1535, TA97a, TA98, TA102, and TA100, at concentrations of from 8 to 5000 ug/plate (based on precipitation at 5000 ug/plate and bacteriotoxicity at ≥1250 ug/plate) in the presence and absence of S-9 mix (incubation at 37C for 3 days). Three experiments were performed. Experiment 1 was conducted by the preincubation method (20 min at 37°C) and experiment 2 was conducted without preincubation as a plate incorporation test. In experiment 1, no bacteriotoxicity was only seen at ≥5000 ug/plate for 3 strains, but for 2 strains (TA100 and TA102), no background growth was seen at concentrations >8 ug/plate, indicating an invalid experiment. A third experiment was conducted only in these 2 strains, and bacteriotoxicity was then seen at ≥1250 ug/plate. Although some small increases in the number of revertant colonies were seen in TA100 in experiments 2 and 3, no clear effects were produced. Appropriate control responses were observed.

C. CHINESE HAMSTER OVARY CLASTOGENICITY TEST WITH SYNTHESIS 2 GP 47680 (Tox. Ref. 3-16, Test no. 896309, conducted by [redacted] 1990, GLP, Vol. 1.67)

The potential of GP 47680 (Batch no. 800189, in DMSO) to induce chromosomal aberrations in cultured CCL-61 Chinese hamster ovary cells was evaluated at concentrations of from 3.9 to 500 ug/ml, with (3 hr trmt/15 or 39 hr recovery) and without (18 or 42 hr trmt) metabolic activation. In the toxicity test, there was marginal or no suppression of mitotic activity at up to 500 ug/ml, which was considered "the highest applicable concentration." There were no increases in aberrations in the experiments (original and confirmatory) performed with metabolic activation or without activation and with an 18 hr treatment time, but in the experiment performed without activation and with a 42 hr treatment time, the number of cells with specific aberrations was concentration-dependently increased at concentrations of 15.63 ug/ml or greater and the frequency of aberrations was outside the control range at 31.25 ug/ml (**Table VC**). In addition, an increase in numerical chromosomal aberrations (polyploidy) was seen at concentrations of 7.81 ug/ml or greater. Concentrations ≥62.5 ug/ml displayed predominantly polyploid metaphases. Appropriate positive control responses were seen in some of the experiments (1 and 2), but according to the report, "the analysis of positive controls from the third and fourth experiment of the confirmatory study was omitted for practical reasons."

Table VC

The effect of GP 47 680 on Chinese hamster ovary cells in vitro without metabolic activation

Experiment 3 Confirmatory study	Treatment 42h			
	Vehicle	GP 47 680		
	Control	7.81	15.63	31.25 ug/ml
Percent of metaphases with specific aberrations	0	3.5	4.5	7.5
Metaphases with				
Chromatid breaks			1	5
Iso-chromatid breaks				
Deletions				
Iso-chromatid-deletions				
Chromatid exchanges		1	2	5
Di-, polycentrics		2	3	5
Ring chromosomes		1		1
Acentric rings				
Chromatid fragments				
Iso-chromatid fragments		3	5	3
Percent of metaphases with unspecific aberrations	2	5.5	3	8.5
Metaphases with				
Chromatid gaps	4	10	6	12
Iso-chromatid gaps		1		
Chromosome decay (part.)				4
Chromosome decay (compl.)				
Premature Chromosome Condensation (PCC)				1
Number of polyploid metaphases in addition to 200 metaphases with normal chromosome number	1	13	36	181

200 metaphases per concentration scored

D. V79/HGPRT CHINESE HAMSTER CELL GENE MUTATION TEST WITH SYNTHESIS 2 GP 47680
(Tox. Ref. 3-17, Test no. 896310, conducted by [redacted]/1990, GLP, Vol. 1.67)

The mutagenic potential of GP 47680 (Batch no. 800189, in DMSO) in cultured V79 Chinese hamster cells was evaluated at concentrations of from 25 to 500 ug/ml with metabolic activation (5 hr trmt; no cytotoxicity limit, 500 ug/ml considered "highest applicable concentration") and from 2.5 to 100 ug/ml without metabolic activation (21 trmt; concentration limited by cytotoxicity). There were no increases in mutation frequencies (6-TG resistant colonies) in these experiments (original and confirmatory). Appropriate positive control responses were observed.

E. V79 CHINESE HAMSTER CELL CHROMOSOMAL ABERRATION TEST WITH SYNTHESIS 2 GP 47680 (Tox. Ref. 3-18, Test no. 971801, conducted by Novartis 1998, GLP, Vol. 1.67)

The clasotogenic potential of GP 47680 (Batch no. 400697, ir DMSO) in cultured V79 Chinese hamster cells was evaluated at concentrations of from 171 to 500 ug/ml with (3 hr trmt/17 or 41 hr recovery) and from 5 to 500 ug/ml without (20 or 44 hr continuous trmt or 3 hr trmt/17 hr recovery) metabolic activation. The high concentrations were based on changes in cell morphology and reduced cell growth at ≥224.5 ug/ml in the absence of metabolic activation and ≥320.0 ug/ml in the presence of activation. Aberration frequencies were increased somewhat compared to C in several experiments, both with and without activation (**Tables VE.1-2**) and in some cases were just outside the historical control range (0-2.5% abnormal cells with S9, 0-3.0% without S9), although the effect was not great or concentration related. In addition, frequencies of polyploidy were increased at concentrations of ≥5 ug/ml without metabolic activation and ≥171 ug/ml in the presence of metabolic activation. Positive control responses were appropriate.

Table VE.1

Chromosomal aberration analysis after treatment for 3 hours with Trileptal[a] and recovery for 41 hours in the presence of 10% rat liver S9.

	Experiment CA2					
	Cell Growth	Mitotic Index %	% Abn. Cells	% Cells Exch.	Cells analyzed	% cells polyploid
Control						
S9	+ + +	1.8[a]	0.4	0.0	273	2.2
Trileptal[a] (µg/ml)						
500.0	+ +	344.4[a,b]	IA	IA	422	98.6
382.4	+ +	611.1[a,b]	IA	IA	486	99.6
292.4	+ + +	272.2[a,b]	1.5	0.0	783	74.5
223.6	+ + +	355.6[a,b]	IA	IA	1966	93.3
171.0	+ + +	244.4[a,b]	2.0	0.0	623	67.9
130.8	+ + +	177.8[a,b]	ND	ND	ND	ND
100.0	+ + +	172.2[a,b]	ND	ND	ND	ND
Positive Control, CP (µM)						
15.0	ND	ND	14.0	0.0	101	1

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Table VE.2 Chromosomal aberration analysis after treatment for 3 hours with Trileptal[a] and recovery for 17 hours in the absence of 10% rat liver S9.

	Experiment CA3					
	Cell Growth	Mitotic Index %	% Abn. Cells	% Cells Exch.	Cells analyzed	% cells polyploid
Control						
S9	+ + +	6.2*	1.0	0.0	202	2
Trileptal[a] (µg/ml)						
500.0	+ +	125.8**	IA	IA	1099	89.7
303.5	+ +	161.3**	ND	ND	ND	ND
184.2	+ + +	151.6**	ND	ND	ND	ND
111.8	+ + +	87.1**	2.5	0.0	210	4.8
67.9	+ + +	98.4**	ND	ND	ND	ND
41.2	+ + +	96.8**	3.0	0.0	203	1.5
25.0	+ + +	101.6**	ND	ND	ND	ND
Positive Control, EMS (mM)						
12.5	ND	ND	22.0	9.0	102	2

IA: invalid analysis, less than 160 metaphases could be analysed

ND not determined

* Mitotic indices as % of mitotic cells within the total population of mitotic and non-mitotic cells.
** Mitotic indices as % of the controls.

% cells exch.: % cells with exchanges

Cell growth estimated relative to the controls: +++, normal cell density; ++, 100-75% cell density of the control; +, 75-50%; -, 50-25% and --, < 25%.

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F. RAT MICRONUCLEUS TEST WITH (SYNTHESIS 2) GP 47680 (Tox. Ref. 3-19, Test no. 896308, conducted by [redacted] 1990, GLP, Vol. 1.67)

GP 47680 (batch no. 800189) was evaluated for its ability to induce micronucleus formation at 16, 24, and 48 hr after po administration of a dose of 2500 mg/kg (considered MTD) and at 24 hr after po administration of 625, 1250, or 2500 mg/kg to rats (5/sex/grp evaluated at each sacrifice time). There were no significant increases in numbers of micronucleated PCEs in GP 47680 groups, while the positive control produced the expected increase in micronucleated cells.

G. AMES TEST WITH GP 47779 (SYNTHESIS 2) (Tox. Ref. 4-16, Test no. 946130, conducted by [redacted] in 1994, GLP, Vol. 1.73)

When GP 47779 (Batch no. 800294; dissolved in DMSO) was evaluated in tester strains S. typhimurium TA1537, TA1535, TA100, TA102, TA98, and E. coli WP2 uvrA at concentrations ranging from 312.5 to 5000 ug/plate (no toxicity seen at highest concentration) with and without S-9 (incubation at 37C for 48 hr), no increases in incidences of either histidine- or tryptophan-prototrophic mutants were observed compared to the negative control.

H. CHINESE HAMSTER OVARY CELL GENE MUTATION TEST WITH GP 47779 (SYNTHESIS 2) (Tox. Ref. 4-17, conducted at [redacted] 1990, non-GLP, Vol. 1.73)

The potential of GP 47779 (Batch unspecified, in DMSO) to induce mutations at the HGPRT locus

in CHO cells in vitro was evaluated in presence and absence of S9 at concentrations of from 1 to 8 mg/ml with a 1 hr treatment time and from 0.5 to 4 mg/ml with a 4 hr treatment time. Concentrations were based on the results of a cytotoxicity test in which 2 mg/ml (1 hr trmt) reduced survival to 69% and 4 mg/ml reduced survival to 29.9% (**Figures VH**). Survival was markedly reduced at the next doses tested: to 1.5% at a concentration of 6 mg/ml. The addition of S9 had no effect on cytotoxicity. In the mutagenicity tests, there were no increases in mutation frequencies at concentrations of up to 2 mg/kg; however, increased mutant frequencies compared to C were seen at a cytotoxic concentration of 4 mg/ml, with and without metabolic activation (**Table VH**). Appropriate positive control responses were observed.

Figure VH Toxicity test of GP 47779 in CHO cells following 1 hr treatment

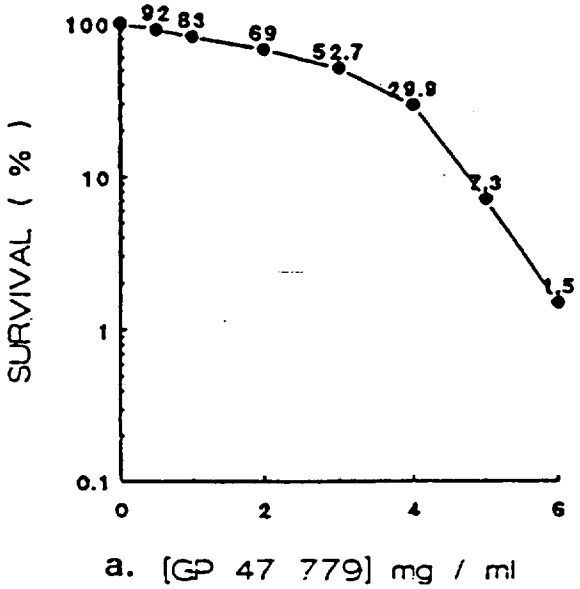

Table VH Detection of gene mutation at the HGPRT-locus in CHO 9 cells following 1 treamtment with GP 47 779

Compound	Concentration mg/ml	µM	S9-mix	Frequency of TG^r mutants/10^6 viable cells	Induced TG^r muta 10^6 viable cells
GP47 779					
	0		-	1.5	-
	0		+	1.5	-
	1		-	1.3	-
	1		+	1.5	-
	2		-	1.4	-
	2		+	1.5	-
	4		-	16.6	15.1
	4		+	17.8	16.3
DMBA (1h treatment)					
		10	-	3	-
		10	+	245	242

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I. V79 CHINESE HAMSTER CELL GENE MUTATION TEST WITH (SYNTHESIS 2) GP 47779 (Tox. Ref. 4-18, Test no. 916088, conducted by [redacted] 1991, GLP, Vol. 1.73)

The mutagenic potential of GP 47779 (Batch no. 800188, in DMSO) in cultured V79 Chinese hamster cells was evaluated at concentrations of from 74.07 to 2000 ug/ml (no cytotoxicity at highest concentration; 2000 ug/ml considered "highest applicable concentration"), with and without metabolic activation (4 hr trmt). There were no increases in mutation frequencies (6-TG resistant colonies) under these conditions (original and confirmatory). Appropriate positive control responses were observed.

J. CHINESE HAMSTER OVARY CLASTOGENICITY TEST WITH (SYNTHESIS 2) GP 47779 (Tox. Ref. 4-14, Test no. 906203, conducted by [redacted] 1990, GLP, Vol. 1.73)

The potential of GP 47779 (Batch no. 800188, in DMSO) to induce chromosomal aberrations in cultured CCL 61 Chinese hamster ovary cells was evaluated at concentrations of from 250 to 2000 ug/ml, with (3 hr trmt/15 or 39 hr recovery) and without (18 or 42 hr trmt) metabolic activation. In the toxicity test, the highest concentration in the first experiment, 1000 ug/ml (18 hr w/o activation), produced a 52.1% suppression of mitotic index; the high concentration in the second experiment, 2000 ug/ml (3 hr w/ activation, 15 hr recovery), caused no suppression of mitotic index; the high concentration in the third experiment, 1000 ug/ml (42 hr w/o activation), produced 44.1% suppression; and the high concentration in the fourth experiment, 2000 ug/ml (3 hr w/ activation, 39 hr recovery), caused no suppression of mitotic activity. In experiment 1 (18 w/o activation), the percentages of metaphases with chromosomal aberrations were concentration-dependently increased compared to the negative control (**Table VJ**). In experiment 3 (42 hr w/o activation), aberrations were increased somewhat at all concentrations (4.5, 4.5, and 4.5% specific aberration at 250, 500, and 1000 ug/ml, respectively, compared to 3.5% in C). In experiment 4 (3 hr w/ activation/39 hr recovery), aberrations

were also increased by GP 47779, but not in a concentration-dependent manner (5.0, 1.0 and 4.0% specific aberration at 500, 1000, and 2000 ug/ml, respectively, compared to 1.5% in C). In addition, an increase in numerical chromosomal aberrations (polyploidy) was seen at the high concentration in experiment 3 (17 vs 3 in neg control). There were no clear effects in experiment 2. Appropriate positive control responses were observed

Table VJ.

The effect of GP 47 779 on Chinese hamster ovary cells in vitro without metabolic activation

Experiment 1 Treatment 18h

	Vehicle Control	GP 47 779 250.0	500.0	1000.0 µg/ml	Pos. Control * Mitomycin-C 0.2 µg/ml
Percent of metaphases with specific aberrations	3	3.5	5	7.5	20
Metaphases with					
Chromatid breaks	2	2	6	6	6
Iso-chromatid breaks				1	
Deletions					
Iso-chromatid-deletions				1	
Chromatid exchanges					3
Di-, polycentrics	2			1	
Ring chromosomes		2	1		
Acentric rings			1		
Chromatid fragments	1	2		2	2
Iso-chromatid fragments	1	2	4	4	1
Percent of metaphases with unspecific aberrations	4	5	5.5	8	14
Metaphases with					
Chromatid gaps	8	8	11	15	6
Iso-chromatid gaps		2	1	1	1
Chromosome decay (part.)					
Chromosome decay (compl.)					
Premature Chromosome Condensation (PCC)	1				

200 metaphases per concentration scored

K. RAT MICRONUCLEUS TEST WITH (SYNTHESIS 2) GP 47779 (Tox. Ref. 4-15, Test no. 916183, conducted by [redacted] 1991, GLP, Vol. 1.73)

GP 47779 (batch no. 800188) was evaluated for its ability to induce micronucleus formation at 16, 24, and 48 hr after po administration of a dose of 2960 mg/kg (considered MTD) and at 24 hr after po administration of 740, 1480, or 2960 mg/kg to rats (5/sex/grp evaluated at each sacrifice time). There were no significant increases in numbers of micronucleated PCEs in GP 47779 groups, while the positive control produced the expected increase in micronucleated cells.

VI. REPRODUCTIVE TOXICITY

A. ORAL REPRODUCTION (SEGMENT I) STUDY OF GP47680 (SYNTHESIS 1) IN RATS (Ref 3-1, Study No. 79 1181, conducted by [redacted] in 1980, pre-GLP, Vol 1.64)

1. Methods

Male and female rats (15 males, 30 females/grp) received 0, 25, 50, or 150 mg/kg by oral gavage (suspension in 2% CMC) prior to (60 days for males, 14 days for females) and throughout mating (1M:2F cohabitation, 12 day mating period) and until sacrifice. Females were either sacrificed on day 21 of gestation (1/2) or after delivery and weaning of offspring (PND 28). Males were sacrificed after weaning of offspring. Litter parameters were evaluated at C-section and at birth. Postnatal developmental parameters were assessed in the offspring of dams allowed to deliver, and 1 male/litter or 2 females/litter were randomly selected for mating.

Strain: Tif: RAIf (SPF)
Drug batch #: 1/78

2. Results

a. F0 Data

i. There were no deaths or T-R clinical signs.
ii. There were no effects on BW gain or food consumption in males. Small D-R decreases in BW gain were seen in treated females during pregnancy and lactation (only shown graphically in **Figure VIA.1**, no data provided), and food consumption was decreased somewhat in all groups during gestation (12-18% at HD).
iii. There were no T-R effects on mating index or time to mating, but fertility indices were decreased somewhat at all doses (90, 83, 80, and 83 in C, LD, MD, and HD groups, respectively). Gestation lengths were comparable among groups.

b. Litter Parameters

i. At term sacrifice, resorptions were slightly increased in HD litters and fetal weights were slightly but D-D decreased in MD and HD litters (**Table VIA.1**). Note that corpora lutea were not counted. No increase in abnormalities was reported, but fetuses were only examined externally.
ii. Litter data from dams allowed to deliver indicated no T-R effects on litter size or pup mortality. No external abnormalities were reported. Pup weights appeared to be decreased slightly in MD and HD groups through weaning (data only provided graphically; **Figure VIA.2**).
iii. There were said to be no group differences in the attainment of physical and functional developmental landmarks (pinna unfolding, incisor eruption, eye opening, testes descent, vaginal opening, righting reflex, pupillary reflex, startle reflex), but the data were not provided.
iv. Exploratory behavior expressed as an activity index was reduced slightly (7% compared to C) in HD offspring when tested on PND 32.
v. There were no group differences in offspring reproductive function.

Figure VIA.1

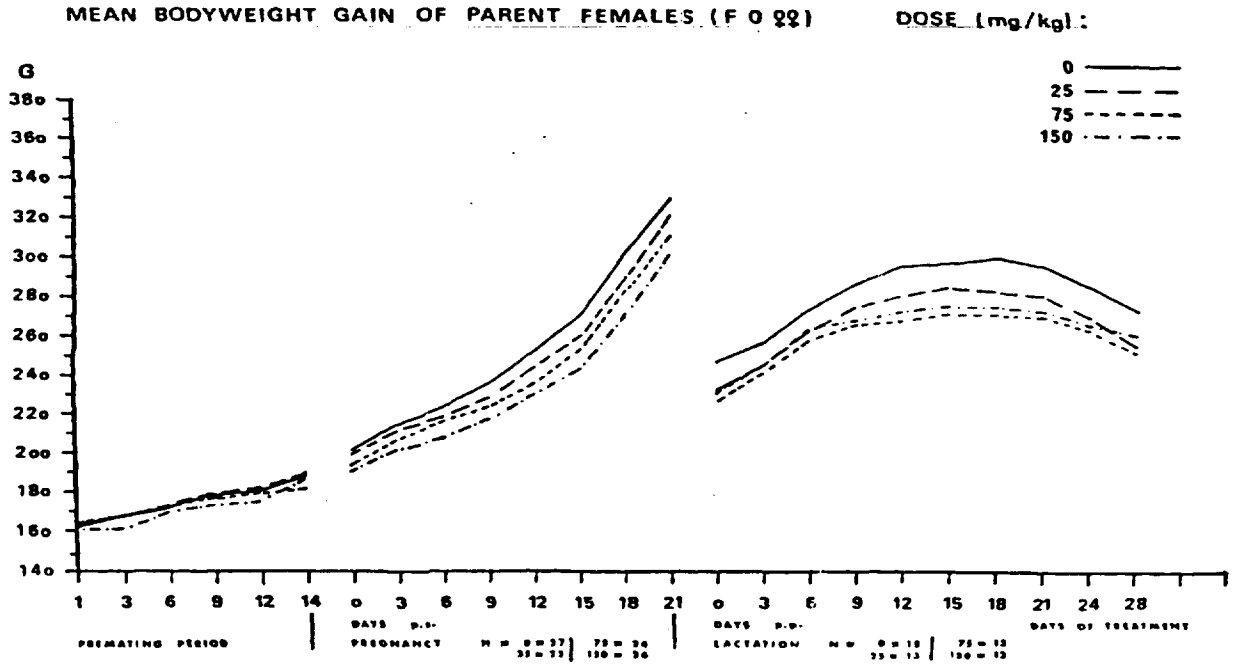
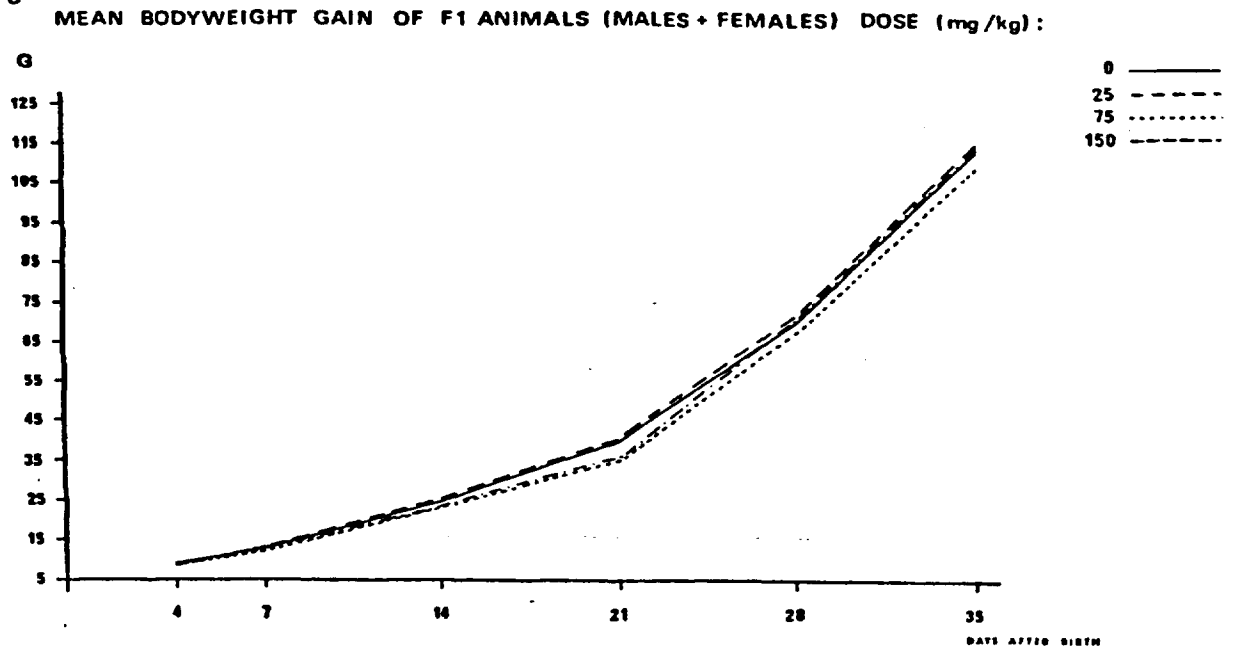


Table VIA.1 Litter data for dams sacrificed on day 21 of gestation

Reproductive parameters	Dose (mg/kg)			
	0	25	75	150
No. pregnant	12/12	12/13	11/14	13/15
Implantations (mean ± SD)	13.0 ± 1.4	12.8 ± 2.0	13.4 ± 1.5	12.6 ± 0.8
Live fetuses (M/F)	81/61	72/65	70/69	70/72
Resorptions - early (%)	9.0	10.5	5.4	12.8
Resorptions - late (%)	0	0	0	0.6
Dead fetuses	0	0	0	0
Fetal body weight (g, mean ± SD)	5.1 ± 0.4	5.1 ± 0.4	4.9 ± 0.4	4.8 ± 0.4

Figure VIA.2



B. ORAL FERTILITY AND EARLY EMBRYONIC DEVELOPMENT STUDY OF GP 47779 (SYNTHESIS 2) IN RATS (Tox Ref 4-4, Study No. 974070, conducted by Novartis in 1998, GLP, Vol. 1.69)

1. Methods

Male and female rats (24/sex/grp) were dosed with 0 (vehicle: aqueous 0.5% CMC), 50, 150, or 450 mg/kg orally by gavage for 28 (males) or 14 (females) days prior to mating, throughout mating (2-week mating period), and through day 6 of gestation in females or for a total of 52 days in males. Females underwent C-section on day 13 of gestation.

Strain: Sprague-Dawley - Crl:CD(SD)BR
Drug lot #: 800494

Dose selection was based on the results of a rat teratology study of GP 47779 (75, 250, and 500 mg/kg), in which maternal toxicity was seen at ≥250 mg/kg, and on the results of the 13-week rat carcinogenicity dose range-finding study of GP 47779 (50, 200, 600, and 2000 mg/kg), in which toxicity was observed at ≥600 mg/kg.

2. Results

a. Clinical observations

There were no deaths. D-R ataxia and salivation were observed at the MD and HD in both sexes.

b. Body weight gain and food consumption

Food consumption was decreased (SS) throughout much of the treatment period in HD males and throughout the premating and early gestation periods in HD females. BW gain over the course of the study was decreased (25% below C; SS) and final BWs were lower

(9%) in HD males. In females , BW gain was transiently (days 0-3) decreased in all groups during the 2-week premating period and was decreased throughout the gestational treatment period in the HD group (25% below C). Corrected gestational weight gain was slightly decreased (3% below C; NS) and corrected GD 13 BW was lower (5%; SS) at the HD (**Table VIB.1**).

c. Fertility and Reproductive parameters

i. Mating and fertility parameters were comparable among groups.
ii. When vaginal cytology examinations were performed during the premating period, the number of acyclic females was increased (5/24 vs 0/24 C) and the number with regular cycles reduced (13 vs 17) in the HD group.
iii. Numbers of corpora lutea, implants, and live embryos were reduced in treated females compared to controls, reaching statistical significance at the HD (**Table VIB.2**). There were no apparent T-R differences in pre- or postimplantation loss.
iv. Testes weights were not affected by treatment, and while sperm analysis suggested possible slight effects on sperm numbers and motility, there were no statistically significant differences among groups for these parameters (**Table VIB.3**).

Table VIB.1 Summary of female corrected body weight (gm; Mean ± SD)

	Dose - mg/kg			
Day	0	50	150	450
13	328.2 ± 23.2 (22)	323.7 ± 19.8 (23)	324.2 ± 21.1 (21)	311.2 ± 18.1** (22)

Table VIB.2 Summary of female reproductive parameters (mean ± SD)

	Dose (mg/kg)			
	0	50	150	450
Number of Litters	22	23	21	21
Corpora Lutea	18.6 ± 2.7	17.0 ± 2.1	18.0 ± 3.4	16.1 ± 2.7*
Uterine Implants	17.1 ± 1.8	15.0 ± 4.5	15.4 ± 2.0*	14.7 ± 2.3**
Live embryos	16.1 ± 1.8	14.3 ± 4.3	15.0 ± 2.1	14.05 ± 2.4*
Resorptions	0.9 ± 1.0	0.7 ± 1.0	0.5 ± 0.7	0.7 ± 0.9
Pre-implantation Loss	1.6 ± 1.9 -	2.0 ± 3.7	2.5 ± 2.7	1.4 ± 1.7
Post-implantation Loss	0.9 ± 1.0	0.7 ± 1.0	0.5 ± 0.7	0.7 ± 0.9

* Significantly different from control, $p \leq 0.05$
** Significantly different from control, $p \leq 0.01$

Table VIB.3

Summary of sperm analysis and male reproductive organ weights
(Mean ± Standard deviation)

Organ	Dose level (mg/kg/day)			
	Control (0)	50	150	450
Terminal body wt (gms)	530.46 ± 46.10 (24)	532.67 ± 39.67 (24)	533.67 ± 42.77 (24)	484.17 ± 34.88** (24)
Testes weight (gm)	3.35 ± 0.23 (24)	3.53 ± 0.33 (24)	3.47 ± 0.24 (24)	3.50 ± 0.28 (24)
Testes/body weight (%)	0.64 ± 0.06 (24)	0.66 ± 0.06 (24)	0.66 ± 0.07 (24)	0.73 ± 0.06** (24)
Epididymal weight (gm)	1.45 ± 0.09 (24)	1.53 ± 0.14 (24)	1.45 ± 0.10 (24)	1.40 ± 0.12 (24)
# Sperm ($x10^5$)/gm testes	87.05 ± 14.24 (24)	83.36 ± 18.61 (24)	81.99 ± 12.14 (24)	82.06 ± 17.49 (24)
% motility	83.34 ± 5.49 (24)	84.34 ± 9.47 (24)	81.80 ± 15.60 (24)	80.02 ± 13.59 (24)

C. ORAL TERATOGENICITY (SEGMENT II) STUDY OF GP 47680 (SYNTHESIS 1) IN MICE (Tox. Ref. 3-2, Exp. no. 32 74 10 00, conducted by [redacted] in 1974, pre-GLP, Vol. 1.64)

1. Methods

Mated female mice (30/grp) were given 0 (vehicle:CMC), 25, 75, or 250 mg/kg on gestation days 6 through 15 by oral gavage. On day 18, C-sections were performed on all dams; numbers of live, dead, and resorbed fetuses were recorded; live fetuses were weighed, sexed, and examined for external abnormalities; and fetuses from each litter were examined for visceral (1/3, Wilson's method) or skeletal (2/3, Dawson's) defects.
Strain: NMRI-derived
Drug batch #: 6

2. Results

a. Maternal effects

No maternal deaths occurred. Clinical signs (somnolence, dyspnea, recumbency, ataxia) and decreased food consumption were reported at the HD. There were no effects on maternal BW gain, however.

b. Fetal evaluations (**Table VIC.1**)

The only clearly T-R developmental effect was a small decrease (6%; SS) in fetal weights in HD litters. However, the only malformations reported were in the HD group, where 2 fetuses with agnathia were found (no indication of affected litters).

Table VIC.1. Summary of litter data (means) from mouse teratology study of GP 47690

Reproductive parameters	Dose (mg/kg)			
	0	25	75	250
No. pregnant	25	22	27	25
Implantations	11.6	11.5	11.4	11.2
Live fetuses (%)	9.8 (88.2)	10.5 (91.3)	10.2 (89.3)	9.8 (88.2)
Early resorptions (%)	1.2 (11.1)	0.8 (6.7)	1.1 (9.4)	1.4 (11.9)
Late resorptions (%)	0.1 (0.7)	0.2 (1.5)	0.2 (1.3)	0.2 (1.4)
Dead fetuses (%)	0	1 (0.1)	0	0
Fetal body weight (g, mean ± SD)	1.09 ± 0.15	1.09 ± 0.13	1.08 ± 0.13	1.03 ± 0.13**
No. live fetuses malformed	0	0	0	2

D. ORAL EMBRYO-FETAL DEVELOPMENT STUDY OF GP 47680 (SYNTHESIS 2) IN RATS (Tox. Ref. 3-7, Study no. MIN 941020, conducted by [redacted] in 1994 (pre-ICH), GLP, Vol. 1.65)

1. Methods

Assumed pregnant female rats (26/grp) were given oral (gavage) doses of 0 (vehicle=0.5% CMC), 30, 300, or 1000 mg/kg on days 6 through 15 of gestation. Cesarean sections were performed on day 20 of gestation. The following parameters were evaluated: uterine weights; numbers of corpora lutea, implantations, resorptions, live and dead fetuses; fetal weights; and external (all fetuses), visceral (1/3), and skeletal (2/3) fetal structural abnormalities (not 50:50 as per ICH; method not described).

Strain: Sprague-Dawley (Crl:COBS CD(SD)BR)
Drug batch #: 000692

Dose selection was based on the results of a previous rat teratology study of Synthesis 1 GP 47680 (30, 100, and 300 mg/kg), in which slight maternal and embryotoxicity were seen at ≥100 mg/kg and increased embryonic death occurred at 300 mg/kg, and on the results of the 13-week rat carcinogenicity dose range-finding study with the new batch of GP 47680 (100, 300, 1000, and 3000 mg/kg), in which toxicity was observed at ≥300 mg/kg and moribundity occurred at ≥1000 mg/kg.

2. Results

a. Effects on the dam

One T-R death occurred at the HD (GD 17). Clinical signs were reported at the MD (ataxia, hypoactivity) and HD (hypoactivity, hyperactivity, ptosis, ataxia, chromodacryorrhea, fecal changes). Food consumption and BW gain were significantly reduced throughout treatment in HD dams and were transiently (GD 6-8)

reduced at the MD (**Table VID.1**). Corrected GD 0-20 BW gains were significantly lower in MD (15%) and HD (65%) dams compared to C.

b. Fetal evaluations

i. Resorptions (all early) were increased (4-fold C; SS) in HD litters (**Table VID.2**). Fetal body weights were markedly decreased in HD litters (30% below C; SS); mean weights were 3.81, 3,78, 3.72, and 2.62 gm in males and 3.64, 3.63, 3.52, and 2.53 gm in females in C, LD, MD, and HD groups, respectively.

ii. Fetal and litter incidences of malformations were increased (SS) in MD and HD litters (**Table VID.3**). Gross malformations were found only at the MD (1/366 fetuses, 1/24 litters) and HD (2/252 fetuses, 2/19 litters). At the MD, 1 fetus (#10, dam # 78) had gastroschisis. At the HD, one fetus (#3, dam #119) had agnathia, while another (#1, dam #117) had multiple associated craniofacial malformations including aglossia and astomia. Visceral malformations were also found only in MD (5/116, 3/24) and HD (4/78, 4/19) fetuses/litters. At the MD, 4 fetuses from 2 litters (#s 3, 12, and 15 from dam #61 and #15 from dam # 76) had misshapen eyes and 1 fetus from another litter (#9, dam #71) had dilated brain ventricles. At the HD, 1 fetus (#11, dam #117) had microphthalmia, cleft palate, and heart malformations (irregular heart, septal defect, fibrotic ventricle). Another fetus (#3, dam #119) also had misshapen eyes, cleft palate, and heart malformations (irregular heart, persistent truncus arteriosus, enlarged), while a 3rd fetus (#6, dam # 94) had only microphthalmia and a 4th (#11, dam # 103) had cleft palate only. Skeletal malformations were found only in the HD group (3/174, 3/19). These included fused occipitals and agenesis, fused, and/or misaligned vertebrae/centra. Individual data did not indicate any correlation between maternal toxicity and malformations.

iii. Incidences of visceral and skeletal variations were increased at the MD and HD (**Table VID.3**). At the MD, a fetus (#9, dam # 71) with a visceral malformation also had coagulated blood in the brain, which was considered a visceral variation. At the HD, 1 fetus with multiple malformations (#3, dam # 119) also had thickened liver, lung alterations, and situs inversus of the abdominal organs, while another (#11, Dam # 117) had a reduced lung size. A variation termed irregular liver was found in 2 additional HD fetuses (#6, dam #119; #3, dam #71). In addition, ossification of various skeletal elements was reduced (SS) in MD and HD litters.

Table VID.1 Summary of maternal body weight gain (gm) in rat teratology study of GP 47680

(MEAN ± SD)

DAYS	DOSE LEVEL (MG/KG/DAY)			
	CONTROL (0)	30	300	1000
0-6	36.16 ± 10.22 N = 25	39.08 ± 8.10 N = 26	42.75 ± 7.39 N = 24	37.88 ± 11.39 N = 24
6-8	8.40 ± 8.12 N = 25	8.85 ± 5.62 N = 26	-15.71 ± 10.24** N = 24	-25.08 ± 11.81** N = 24
8-12	27.72 ± 5.87 N = 25	27.08 ± 5.87 N = 26	27.00 ± 11.90 N = 24	-5.75 ± 11.38** N = 24
12-16	29.32 ± 7.14 N = 25	31.42 ± 7.84 N = 26	32.96 ± 6.32 N = 24	14.17 ± 22.19* N = 24
16-20	74.60 ± 10.54 N = 25	72.19 ± 14.75 N = 26	74.71 ± 8.73 N = 24	56.39 ± 20.65** N = 23

IN CASES OF STATISTICAL SIGNIFICANCE, '*' FOR .01 < P <= .05, '**' FOR P <= .01

Table VID.2 Summary of reproductive parameters (mean ± SD) in rat teratology study of GP 47680

	Dose (mg/kg)			
	0	30	300	1000
No. pregnant	25	26	24	24
Corpora Lutea	18.2 ± 3.1	19.5 ± 3.3	19.0 ± 3.1	19.8 ± 2.4
Implantation sites	16.4 ± 2.1	16.1 ± 4.0	15.9 ± 1.7	16.7 ± 2.2
Early resorption	1.3 ± 1.1	1.0 ± 1.3	0.6 ± 0.9	5.8 ± 6.3**
Late resorptions	0.1 ± 0.2	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
Total resorptions	1.4 ± 1.2	1.0 ± 1.3	0.6 ± 0.9	5.8 ± 6.3**
Live fetuses	15.1 ± 2.2	15.1 ± 3.9	15.3 ± 1.7	10.96 ± 5.9**
Dead fetuses	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
Postimplantation loss	1.4 ± 1.2	1.0 ± 1.3	0.6 ± 0.9	5.8 ± 6.3**
% postimplantation loss	8.2 ± 7.0	5.5 ± 7.6	3.8 ± 5.3	33.7 ± 34.0**

** $p \leq 0.01$, compared to C

Table VID.3 Summary of fetal examinations (by fetus) in rat teratology study of GP 47680 (sponsor's table)

Type/Parameter	Dose Level (mg/kg/day)			
	Control (0)	30	300	1000
GROSS VARIATIONS:				
Hematoma	0	0	1	0
Total No. of Fetuses with Gross Variations:	0	0	1	0
Total No. of Fetuses Examined Grossly:	377	393	366	252
GROSS MALFORMATIONS:				
Absence of eye bulge	0	0	0	1[a]
Aglossia	0	0	0	1[a]
Agnathia	0	0	0	1[a]
Astomia	0	0	0	1[a]
Ears placed lower than normal	0	0	0	1[a]
Elongated snout	0	0	0	1[a]
Gastroschisis	0	0	1	0
Total No. of Fetuses with Gross Malformations:	0	0	1	2*
Total No. of Fetuses Examined Grossly:	377	393	366	252
VISCERAL VARIATIONS:				
Brain - Coagulated blood	0	0	1	0
Liver - Irregular	0	0	0	2*
Thickened	0	0	0	1
Lung - Additional lobe	0	0	0	1
Irregular	0	0	0	1
Reduced	0	0	0	1
Surface rough	0	0	0	1

Renal papilla - Absent	1	1	2	2
Short	23	22	23	15
Situs inversus	0	1	0	1
Snout - Coagulated blood	0	0	0	1
Ureter - Dilated	5	8	12	4
Total No. of Fetuses with Visceral Variations:	24	25	28	19
Total No. of Fetuses Examined Viscerally:	117	124	116	78
VISCERAL MALFORMATIONS:				
Brain - Dilated ventricles	0	0	1	0
Cleft palate	0	0	0	3*
Heart - Enlarged	0	0	0	1
Fibrotic ventricles	0	0	0	1
Irregular	0	0	0	2*
Septal defect	0	0	0	1
Truncis communis	0	0	0	1
Microphthalmia	0	0	0	2*
Misshapen eyes	0	0	4	1
Total No. of Fetuses with Visceral Malformations:	0	0	5*	4**
Total No. of Fetuses Examined Viscerally:	117	124	116	78

TYPE/PARAMETER	DOSE LEVEL (MG/KG/DAY) CONTROL (0)	30	300	1000
SKELETAL MALFORMATION :				
SKULL				
OCCIPITALS - FUSED	0	0	0	1
CENTRUM/VERTEBRAE				
AGENESIS	0	0	0	1
FUSED	0	0	0	1
MISALIGNED	0	0	0	2 *
NO. FETUSES WITH SKELETAL MALFORMATIONS:	0	0	0	3 *
NO. FETUSES EXAMINED SKELETALLY	260	269	250	174
SKELETAL VARIATION :				
SKULL				
FRONTALS - NOT COMPLETELY OSSIFIED	1	0	0	14
HYOID - NOT COMPLETELY OSSIFIED	30	41	21	5
HYOID - NOT OSSIFIED	3	7	2	3
INTERPARIETALS - NOT COMPLETELY OSSIFIED	48	51	40	93 **
MAXILLAE - NOT COMPLETELY OSSIFIED	2	2	0	0
NASALS - NOT COMPLETELY OSSIFIED	0	0	0	14 **
OCCIPITALS - NOT COMPLETELY OSSIFIED	48	56	28	93 **
PALATINES - NOT COMPLETELY OSSIFIED	0	0	0	2
PARIETALS - NOT COMPLETELY OSSIFIED	6	7	5	41 *
PRESPHENOID - NOT COMPLETELY OSSIFIED	1	2	1	17 **
PRESPHENOID - NOT OSSIFIED	1	1	0	18
SQUAMOSALS - NOT COMPLETELY OSSIFIED	8	4	6	1
TEETH - NOT OSSIFIED	1	11	17 *	96 **
ZYGOMAS - NOT COMPLETELY OSSIFIED	4	4	8	0
CENTRUM/VERTEBRAE				
ADDITIONAL	0	0	0	1
BIPARTITE	4	3	5	10 *
CERVICAL RIB	0	0	1	4 *

IRREGULAR SHAPE	0	0	1	0
NOT COMPLETELY OSSIFIED	46	72	64	119 **
NOT OSSIFIED	4	11	8	79 **
14TH RUDIMENTARY RIB	26	41	26	22
RIBS				
LOCALIZED THICKENING	1	0	0	0
NOT COMPLETELY OSSIFIED	0	0	0	1
RUDIMENTARY RIB	2	0	0	0
WAVY/ANGULATED	2	2	0	0
STERNEBRAE				
BIPARTITE	0	1	0	6 **
IRREGULAR SHAPE	32	29	30	33
NOT COMPLETELY OSSIFIED	172	190	192	125
NOT OSSIFIED	64	88	119 **	165 **
FORELEG/FOREPAW				
DISTAL PHALANGES - NOT OSSIFIED	0	1	0	20 **
METACARPALS - NOT COMPLETELY OSSIFIED	104	102	57	31
METACARPALS - NOT OSSIFIED	117	130	139	166 **
PELVIC GIRDLES				
ISCHIUM - NOT COMPLETELY OSSIFIED	0	0	0	4
ISCHIUM - NOT OSSIFIED	0	0	0	2
OS PUBIS - NOT COMPLETELY OSSIFIED	5	6	5	30 **
OS PUBIS - NOT OSSIFIED	0	2	0	32 **
HINDLEG/HINDPAW				
DISTAL PHALANGES - NOT OSSIFIED	0	1	0	41 **
METATARSALS - NOT COMPLETELY OSSIFIED	1	4	2	29 **
METATARSALS - NOT OSSIFIED	0	2	0	60 **

* $p \leq 0.05$, ** $p \leq 0.01$, compared to C

E. ORAL TERATOGENICITY (SEGMENT II) STUDY OF GP 47779 (SYNTHESIS 2) IN RABBITS (Tox. ref. 4-7, Report no. T/P (US) 95036, conducted by [redacted] in 1994 (pre-ICH), GLP, Vol. 1.70)

1. Methods

Presumed pregnant female rabbits (20/grp) were given oral (gavage) doses of 0 (vehicle=0.5% CMC), 20, 100, or 200 mg/kg on days 7 through 19 of gestation. Maternal mortality, clinical signs, food consumption, and body weight were recorded. Cesarean sections were performed on day 29 of gestation. The following parameters were evaluated: uterine weights; numbers of corpora lutea, implantations, resorptions, live and dead fetuses; fetal weights; and external, visceral, and skeletal fetal structural abnormalities (all fetuses).

Strain: New Zealand White (Hra:(NZW)SPF)
Drug lot #: 800594

Dose selection was based on the results of a dose range-finding study (150, 500, and 1000 mg/kg lowered to 75, 150, and 250 mg/kg after the first dose due to convulsions at ≥500 mg/kg), in which mild maternal toxicity (clinical signs, transient decrease in BW gain) occurred at 250 mg/kg. No developmental effects were observed in this study.

2. Results

a. Maternal effects

There were no T-R deaths during the study. Clinical signs (splayed limbs, limb spasms) and slightly decreased BW gain during the treatment period were observed at the HD (**Table VIE.1**). Corrected GD 29 BWs and BW gains were not different among groups. PK parameters determined after oral administration of a dose of 200 mg/kg in a separate group of 7 pregnant rabbits are shown in **Table VIE.2**.

b. Developmental effects

i. Resorptions (early and late) were increased in all treatment groups (total SS at all doses compared to C; **Table VIE.3**). Percent postimplantation loss was 10-fold C at the HD, but the LD and MD effects were small and not D-R and did not result in a decrease in live fetuses.

ii. Fetal body weights were decreased slightly in MD and HD females (both 3% below C; NS).

iii. There were no apparent effects on fetal structure; incidences of malformations and variations were similar among groups (**Table VIE.3**). It should be noted that because of increased resorption fewer fetuses were evaluated at the HD compared to other groups. However, the number of litters evaluated was adequate.

Table VIE.1 Summary of maternal body weight gain (gm) in rabbit teratology study of GP 47779

DAY	DOSE LEVEL (MG/KG/DAY)			
	CONTROL (0)	20	100	200
0-7	0.17 ± 0.07 N = 20	0.14 ± 0.08 N = 20	0.13 ± 0.08 N = 18	0.16 ± 0.07 N = 19
7-10	-0.00 ± 0.04 N = 20	0.01 ± 0.02 N = 20	-0.02 ± 0.04 N = 18	-0.09 ± 0.05** N = 19
10-14	0.08 ± 0.04 N = 20	0.07 ± 0.04 N = 20	0.06 ± 0.05 N = 18	0.06 ± 0.07 N = 19
14-20	0.12 ± 0.08 N = 20	0.10 ± 0.05 N = 20	0.11 ± 0.05 N = 18	0.04 ± 0.12 N = 18
20-24	0.08 ± 0.05 N = 20	0.08 ± 0.03 N = 20	0.08 ± 0.04 N = 18	0.14 ± 0.08** N = 18
24-29	0.10 ± 0.07 N = 20	0.10 ± 0.10 N = 20	0.08 ± 0.08 N = 18	0.09 ± 0.07 N = 18
0-29	0.55 ± 0.18 N = 20	0.51 ± 0.15 N = 20	0.44 ± 0.14 N = 18	0.39 ± 0.21* N = 18

IN CASES OF STATISTICAL SIGNIFICANCE, '*' FOR .01 < P <= .05, '**' FOR P <= .01

Table VIE.2. Pharmacokinetic parameters for GP 47779 (MHD), CGP 1000 (DHD), and GP 47680 (OXC) on gestation day (GD) 20 in pregnant rabbits administered 200 mg/kg/day GP 47779 on GDs 16-20

Analyte	Cmax (ug/ml)	AUC (0-24 h) (ug.h/ml)	tmax (h)
MHD	68.4 ± 14.4	270 ± 20	0.5
DHD	5.0 ± 1.0	38.4 ± 6.7	2
OXC	4.2 ± 0.5	17.0 ± 2.6	2

Table VIE.3. Summary of reproductive parameters (mean ± SD) in rabbit teratology study of GP 47779

	Dose (mg/kg)			
	0	20	100	200
No. pregnant	20	20	18	19
Corpora Lutea	12.4 ± 2.6	12.4 ± 1.7	12.2 ± 3.0	11.1 ± 2.6
Implantation sites	8.6 ± 3.3	9.4 ± 2.1	9.3 ± 2.7	8.6 ± 3.0
Early resorption	0.2 ± 0.4	0.6 ± 0.9	0.3 ± 0.5	0.9 ± 1.1**
Late resorptions	0.0 ± 0.0	0.2 ± 0.4	0.2 ± 0.4	0.7 ± 1.7
Total resorptions	1.2 ± 0.4	0.8 ± 1.0*	0.5 ± 0.6*	1.6 ± 1.9**
Live fetuses	8.4 ± 3.2	8.6 ± 2.3	8.8 ± 2.7	6.9 ± 3.5
Dead fetuses	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
Postimplantation loss	0.2 ± 0.4	0.8 ± 1.0*	0.5 ± 0.6*	1.6 ± 1.9**
% postimplantation loss	2.1 ± 4.5	8.6 ± 12.1*	5.6 ± 7.3*	20.1 ± 22.4**
Fetal body weight - grams				
Male	45.0 ± 1.2	45.7 ± 1.2	44.7 ± 1.3	45.1 ± 1.3
Female	44.4 ± 1.2	44.1 ± 1.2	43.2 ± 1.3	43.2 ± 1.4
Malformations				
No. fetuses (litters) examined	168 (20)	172 (20)	158 (18)	125 (18)
No. with external malformations	2 (2)	0 (0)	0 (0)	0 (0)
No. with visceral malformations	2 (2)	2 (2)	0 (0)	0 (0)
No. with skeletal malformations	2 (2)	1 (1)	2 (2)	0 (0)

* $p \leq 0.05$, compared to C
** $p \leq 0.01$, compared to C

F. PRE- AND POSTNATAL DEVELOPMENT STUDY OF GP 47779 (SYNTHESIS 2) IN RATS (Tox. Ref. 4-11, Study no. 974069, conducted by Novartis in 1998, GLP, Vol. 1.72)

1. Methods

Pregnant rats (25/group) were treated with 0 (vehicle: 0.5% CMC), 25, 75, or 250 mg/kg orally (gavage) from gestation day 6 through lactation day 20. The dams were allowed to deliver and rear offspring. F1 offspring were evaluated for survival, growth, physical and behavioral development, and reproductive performance. Dose selection was based on the results of an early (1979) rat teratology study of Synthesis 1 GP 47779 (oral doses of 75, 250, or 500 mg/kg on GD 6-15) in which maternal (decreased BW gain) and developmental (decreased fetal BW and ossification) toxicity were seen at ≥250 mg/kg.

Strain: Sprague Dawley (Crl:COBS CD(SD)BR)
Drug lot #: 800494

2. Results

a. Effects on the dam

1 MD dam died after an apparent dosing accident, but there were no deaths considered T-R. There was no explanation for the apparent D-R decrease in pregnant dams (23, 23, 20, and 19. Clinical signs (salivation, ataxia, tremors) were observed and gestational BW gain was decreased at the HD (30% below C; **Table VIF.1**). Salivation was also noted at MD and a transient effect on gestational BW gain occurred at all doses. Weight gain was increased in HD dams during lactation so that final BWs were similar to controls. Gestation length was increased in the HD group (23.47 vs 23.04 days in C; SS), but other reproductive parameters (postimplantation loss, viable newborn) were similar among groups.

b. Offspring evaluations

i. Litter sizes and pup survival indices were comparable between treated and control groups (**Table VIF.2**).

ii. Pup weights were decreased in HD litters, and this deficit persisted into the postweaning period (**Table VIF.3**).

iii. There were no apparent T-R effects on the attainment of pre- and postweaning developmental landmarks (righting reflex, pinna detachment, eye opening, vaginal opening, preputial separation). There were no SS group differences in the limited behavioral evaluations performed on F1 animals (open field motor activity on day 40, passive avoidance on days 26-33). However, female pups showed small but D-R increases on two measures of activity in the open field test (peripheral bean breaks: 880.5, 895.2, 958.2, and 995.5; total beam breaks: 1009.7, 1022.0, 1110.6, and 1124.0, in C, LD, MD, and HD female pups, respectively). F1 fertility was not affected by treatment.

Table VIF.1 Summary of female gestation body weight gain (gm) in rat pre- and postnatal development study

Days	*Dose level (mg/kg/day)*			
	Control (0)	25	75	250
0-6	31.43 ± 8.20 (23)[a]	28.96 ± 9.15 (23)	25.95 ± 7.10 (21)	29.74 ± 7.74 (19)
6-9	13.35 ± 6.65 (23)	7.91 ± 7.24* (23)	1.76 ± 9.89** (21)	-22.89 ± 9.69** (19)
9-12	16.83 ± 7.57 (23)	19.52 ± 5.17 (23)	17.10 ± 7.98 (20)	25.74 ± 12.99** (19)
12-15	14.26 ± 5.89 (23)	12.39 ± 5.98 (23)	14.20 ± 7.79 (20)	15.05 ± 9.34 (19)
15-18	33.22 ± 8.84 (23)	36.57 ± 7.95 (23)	36.90 ± 6.36 (20)	25.89 ± 8.89** (19)
18-20	25.61 ± 8.11 (23)	29.52 ± 5.08 (23)	28.00 ± 10.13 (20)	27.63 ± 10.70 (19)

Table VIF.2 Summary of F1 litter parameters (mean ± SD) in rat pre- and postnatal development study

	Dose (mg/kg)			
	0	25	75	250
No. viable litters	23	23	20	19
Mean litter size (Day 0)	12.13 ± 0.84	13.61 ± 0.48	14.30 ± 0.48	13.32 ± 0.78
Survival indices				
Days 0-4 (precull)	99.38 ± 0.62	99.69 ± 0.31	99.32 ± 0.47	98.14 ± 1.10
Days 4-21(postcull)	99.46 ± 0.54	98.37 ± 1.19	99.38 ± 0.62	99.34 ± 0.66
Days 4-35 (postcull)	98.91 ± 0.75	98.37 ± 1.19	99.38 ± 0.62	99.34 ± 0.66

Table VIF.3 Summary of pup body weights in rat pre- and postnatal development study of GP 47779

Males

Postpartum day	Dose level (mg/kg/day)			
	Control (0)	25	75	250
0	6.43 ± 0.12 (22)[a]	6.62 ± 0.12 (23)	6.32 ± 0.13 (20)	6.34 ± 0.14 (19)
4 (Precull)	9.68 ± 0.22 (22)	9.76 ± 0.21 (23)	9.65 ± 0.22 (20)	9.57 ± 0.23 (19)
4 (Postcull)	9.64 ± 0.24 (22)	9.69 ± 0.23 (23)	9.66 ± 0.25 (20)	9.47 ± 0.26 (19)
7	15.23 ± 0.36 (22)	15.74 ± 0.35 (23)	15.47 ± 0.37 (20)	15.04 ± 0.39 (19)
14	31.42 ± 0.65 (22)	32.62 ± 0.63 (23)	31.70 ± 0.67 (20)	29.12 ± 0.70* (19)
21	51.95 ± 1.09 (22)	53.29 ± 1.06 (23)	52.99 ± 1.14 (20)	47.69 ± 1.18** (19)
28	92.85 ± 1.61 (22)	94.07 ± 1.56 (23)	92.84 ± 1.67 (20)	87.74 ± 1.73* (19)
35	150.97 ± 2.49 (22)	151.89 ± 2.42 (23)	149.04 ± 2.59 (20)	144.18 ± 2.68 (19)

Females

Postpartum day	Dose level (mg/kg/day)			
	Control (0)	25	75	250
0	6.19 ± 0.10 (23)[a]	6.13 ± 0.10 (23)	5.99 ± 0.11 (20)	5.86 ± 0.12 (19)
4 (Precull)	9.37 ± 0.20 (23)	9.10 ± 0.20 (23)	9.16 ± 0.21 (20)	8.95 ± 0.22 (19)
4 (Postcull)	9.44 ± 0.20 (23)	9.06 ± 0.20 (23)	9.20 ± 0.21 (20)	8.96 ± 0.22 (19)
7	14.82 ± 0.30 (23)	14.71 ± 0.30 (23)	14.72 ± 0.32 (20)	14.05 ± 0.33 (19)
14	31.01 ± 0.58 (23)	31.18 ± 0.58 (23)	31.01 ± 0.62 (20)	27.92 ± 0.63** (19)
21	50.42 ± 1.03 (23)	50.66 ± 1.02 (23)	51.12 ± 1.09 (20)	45.38 ± 1.12** (19)
28	84.94 ± 1.52 (23)	85.04 ± 1.51 (23)	85.85 ± 1.61 (20)	79.13 ± 1.66* (19)
35	130.67 ± 1.92 (23)	129.42 ± 1.91 (23)	129.08 ± 2.04 (20)	122.44 ± 2.10** (19)

VII. SPECIAL TOXICITY

A. COMPARATIVE ASSESSMENT OF PROLIFERATIVE (NEOPLASTIC) LIVER CELL LESIONS OBSERVED MICROSCOPICALLY IN RAT CARCINOGENICITY STUDIES WITH CARBAMAZEPINE AND OXCARBAZEPINE (Tox. Ref. 1-29, conducted by [redacted] in 1983, Vol. 1.47)

Increased incidences of neoplastic liver lesions were observed in 2-years rat studies with carbamazepine and oxcarbazepine, but different diagnostic terms were used for the lesions in the 2 studies, which were conducted by different labs (CBZ by [redacted], OXC by [redacted]). Therefore, 2 pathologist [redacted] reexamined the slides from these studies in order to compare the hepatocellular lesions. It was the opinion of these pathologists that the lesions referred to in the CBZ study as hepatomas (incidences: 5/60, 2/60, 3/60, and 6/60 in males; 2/60, 12/60, 12/60, and 27/60 in females at 0, 25, 75, and 250 mg/kg, respectively) and in OXC study as either benign neoplastic nodules (5/60, 7/60, 8/60, and 9/60 in males; 2/60, 7/60, 6/60, and 3/60 in females at 0, 25, 75, and 250 mg/kg, respectively) or malignant hepatocellular carcinomas (2, 1, 6, and 2 in males; 0, 3, 7, and 7 in females) were very similar histologically, and it was proposed that the term liver cell tumor be used for all of them. The distinction between benign neoplastic nodule and malignant hepatocellular carcinoma was considered to be inexact. Furthermore, it was determined that the tumors in both studies showed no signs of invasiveness or metastasis and did not influence survival.

B. PROMOTION STUDY WITH GP 47680 (Tox. Ref. 1-30, conducted by [redacted] in 1984, Vol. 1.47)

The ability of oxcarbazepine to promote the formation of focal proliferative changes in the rat liver was examined in a neonatal rat model. 24 hr after birth, rat pups received an ip injection of either N-nitrosodiethylamine or vehicle, then at 3 weeks were assigned to treatment groups to receive either GP 47680 (2000 ppm in diet), phenobarbital (positive control; 500 ppm), or control diet for up to 8 weeks. Animals were then sacrificed and livers were examined for proliferative changes by staining for gamma-glutamyl-transpeptidase (GGT) activity. The development of GGT-positive foci was enhanced to a comparable degree by both phenobarbital and GP 47680. It was concluded that with respect to the foci enhancing or promoting effects, exposure to 2000 ppm GP 47680 was similar to exposure to 500 ppm phenobarbital.

C. IN VITRO RED BLOOD CELL HEMOLYSIS TEST OF GP 47779 (Tox. Ref. 2-13, Report No. T/P (US) 96016, conducted by [redacted] in 1996, Vol. 1.63)

The potential of GP 47779 to produce hemolysis was tested in vitro using heparinized blood from beagle dogs. No hemolysis was seen with an aqueous solution of 50 mg GP 47779/20 ml.

VIII. SUMMARY AND EVALUATION

PHARMACOLOGY

The anticonvulsant activity of oxcarbazepine was similar to that of carbamazepine in standard animal seizure models. Oxcarbazepine (OXC, GP 47680) and its active 10-monohydroxy metabolite (MHD, GP 47779) protected against electroshock-induced tonic hindlimb extension in rodents with po ED50 values between 13.5 and 20.5 mg/kg (**Table IA.1**). Predominant CNS side effects in mice and rats were sedation, ataxia, and muscle relaxation, followed by hyperexcitability at higher doses. No tolerance development was observed in rats during 4 weeks of oral administration of MHD (up to 30 mg/kg). The therapeutic indices were 4 (OXC) and > 6 (MHD) for sedation (observation test, mice and rats) and 8 (MHD) or 10 (OXC) for motor impairment (rotorod test, mice). Both compounds were less potent in suppressing chemically induced seizures and did not significantly influence rat kindling development. At doses of 50 mg/kg po and 20 mg/kg im and higher, OXC and, to a lesser extent, MHD protected Rhesus monkeys from aluminum-induced focal seizures. The S(+) enantiomer of MHD was somewhat more potent than the R(-) form in the MES test (**Table IA.2**), but the enantiomers appeared to have similar properties overall (comparable anticonvulsant profiles *in vivo*, similar potencies in suppressing epileptiform discharges in rat hippocampal slices, comparable side effects profiles). In contrast to MHD, the other major metabolite of OXC, the dihydroxy derivative (DHD), was without anticonvulsant effect in the MES test in mice up to a dose of 100 mg/kg p.o.

In vitro, OXC and MHD suppressed sustained high-frequency repetitive firing of sodium-dependent action potentials in mouse neurons in cell culture. Both compounds reduced the percentage of neurons capable of sustained action potential firing in a concentration-dependent manner; the EC50 for OXC was 5 x 10(-8) M and that for MHD was 2 x 10(-8) M ($p > 0.05$ vs. OXC). For comparison, the EC50 for carbamazepine was significantly higher (6 x 10(-7) M, $p < 0.001$ vs. OXC and MHD). Limitation of firing by OXC and MHD depended on firing frequency and membrane potential and was enhanced by depolarization; and input resistance and resting membrane potential were not altered by either drug. These findings are indicative of an effect on voltage-sensitive sodium channels. The *in vitro* effect on action potential firing frequency occurred at concentrations below plasma levels of OXC and MHD that protected animals against electroshock and were therapeutically effective in patients. Blockade of penicillin-induced epileptiform discharges in hippocampal slices by MHD and its stereoisomers was diminished when the potassium channel blocker 4-aminopyridine was added to the bath fluid. This indicates that additional mechanisms of action, e.g., an effect on potassium channels, might be important. Both stereoisomers appeared to be approximately equally responsible for the activity of the racemate. MHD (3-100 uM) also produced a dose-dependent inhibition of glutamatergic excitatory postsynaptic potentials (EPSPs) in rat striatum. This effect was thought to involve the modulation of calcium (Ca^{2+}) signals at either the pre- or postsynaptic level. Patch-clamp studies on rat dorsal root ganglia cells showed that up to a concentration of 3 x 10(-4) M, MHD did not significantly interact with L-type calcium currents, whereas OXC diminished them by about 30% at the concentration of 3 x 10(-4) M. In biochemical investigations, no significant interactions with brain neurotransmitter or modulator receptor sites were identified; neither OXC nor MHD significantly inhibited the binding of NMDA, kainate, or quisqualate receptor ligands in rat brain at concentrations up to 10 uM.

ADME

Rat

GP 47680 (OXC)

Based on AUC(0-24 h) values, intact oxcarbazepine (OXC) and its monohydroxy (MHD) and dihydroxy (DHD) derivatives accounted for 45.6%, 3.7% and 0.7%, respectively of the total radioactivity in plasma after iv administration of ^{14}C-OXC (5 mg/kg) to rats (**Table IIB.1**). After oral administration, the compound was rapidly and apparently well absorbed, extensively metabolized, and largely excreted via the bile (79% of dose in bile fistula rats). Unchanged OXC accounted for only about 3% of the dose excreted in urine and bile. The predominant metabolite was the enol-O-glucuronide of OXC (46% of dose in urine and bile). MHD was a

minor metabolite; 0.7% of the dose was recovered as free MHD and 9% as the MHD-glucuronide in bile and urine. The 0-glucuronide of S(+)-MHD predominated over the glucuronide of R(-)-MHD with a ratio of 7:4. A total of 70% of the dose was accounted for in identified metabolite peaks in bile and urine. In single oral dose studies of OXC in male rats (5, 30, and 300 mg/kg), AUCs of unchanged drug increased proportionally to dose at up to 30 mg/kg, but were less than proportional (~25) at 300 mg/kg, indicating decreased absorption at higher doses. The % in vitro serum protein binding in the rat, rabbit, and dog ranged from 57.7 to 65.6% for OXC over the concentration range of 1-10 ug/ml. Binding was somewhat higher in human serum (72.5%).

GP 47779 (MHD)

When ^{14}C-MHD (5 mg/kg) was given iv to rats, intact MHD, DHD and OXC accounted for 31.5, 13.9 and 17.6%, respectively, of the total radioactivity in plasma (**Table IIB.3**). The other (37%) radioactive components in plasma were not identified. After iv dosing, the t1/2 of intact MHD was 41 min in rats. The major route of excretion in the rat was via the urine after oral dosing (60.2% in urine; 39.1% in feces) and via the feces after intravenous dosing (61.0% in feces; 37.4% in urine) with MHD. Presystemic metabolism following oral administration of ^{14}C-MHD in the rat may account for a greater proportion of the dose being excreted in the urine following oral vs. iv dosing. In bile fistula rats, approximately 60% of an oral dose was excreted in the bile. Intact MHD was the major component identified in the urine and accounted for 10-11% of the dose following both oral and iv routes of administration. About 7% of the dose was excreted in the urine as DHD and less than 0.05% as OXC. There was no apparent difference in metabolite patterns between iv and oral dosing in rats. Plasma AUCs of unchanged drug measured in male rats after single oral doses of MHD (5, 30, and 300 mg/kg) increased greater than dose-proportionally, indicating capacity limited metabolism. Systemic exposure was higher for R(-)-MHD than for S(+)-MHD following both single and multiple oral MHD administration, indicating that S(+)-MHD may be preferentially eliminated. The R(-)/S(+) AUC(0-8 h) ratio following a single dose of racemic MHD was 6.8, and this ratio increased to 12.5 after repeated dosing (14 days). In humans, plasma concentrations of the S(+) enantiomer are greater than those of the R(-)-enantiomer by a ratio of about 4:1 following administration of a single oral dose of OXC. The % in vitro serum protein binding in the rat, rabbit, and dog ranged from 27.3 to 27.8% for MHD over the concentration range of 1-10 ug/ml. Binding was somewhat higher in human serum (39.2).

Dog

GP 47680 (OXC)

Following iv administration of ^{14}C-OXC (5 mg/kg) to dogs, intact OXC and the metabolites MHD and DHD accounted for 14.6, 1.7 and 0.9% of the total labeled substances in plasma, based on AUC (**Table IIB.1**); and the main route of excretion was via the urine. After oral administration of ^{14}C-OXC (5 mg/kg) to dogs, absorption was complete (based on AUCs), and renal and fecal excretion were approximately equal. A large percentage of the radioactivity in dog urine following iv and oral administration of labeled OXC (5 mg/kg) was unidentified; only a small fraction (<2.5%) of the dose appeared in urine as OXC, MHD, or DHD. In single oral dose studies of OXC in male dogs (5, 30, and 300 mg/kg), AUCs of unchanged drug increased less than dose proportionally (~40%, respectively) at 300 mg/kg (**Table IIB.5**). Non-linearity at this dose level was attributed to decreased absorption.

GP 47779 (MHD)

After iv administration of ^{14}C-MHD (5 mg/kg) to dogs, MHD, OXC, and DHD accounted for 34.0, 3.8, and 8.1%, respectively, of total radioactivity in plasma (**Table IIB.3**). The remaining radioactive components in plasma were not identified. The t1/2 of intact MHD was 84 min after iv dosing in dogs. The major route of excretion in dogs was via the urine following both oral and iv administration (76.9 and 75.1% of dose, respectively). Intact MHD was the major component excreted in the urine and accounted for 30.8% and 29.0% of the dose following iv and oral administration, respectively. About 7% of the dose was excreted as DHD and less than 1% as OXC. The metabolite profile in urine was similar following both iv and oral dosing. Plasma AUCs of unchanged drug measured in male dogs after single oral doses of MHD (5, 30, and 150 mg/kg)

increased greater than dose-proportionally, and the amount of unchanged drug in urine as a percentage of dose showed a similar increase (**Table IIB.6**), indicating that MHD metabolism is saturated at higher doses in this species. AUCs for the R(-) enantiomer were about 50% higher than for the S(+) enantiomer following iv administration of MHD to dogs, in contrast to the situation in humans administered OXC (AUC S/R≈ 4).

Human

GP 47680 (OXC)

In humans, the metabolism of OXC appears to consist mainly of reduction to MHD and subsequent conjugation with glucuronic acid. Reduction is stereospecific, but in contrast to rats and dogs, the S(+) configuration of MHD is favored over the R (-) form (AUC S/R≈ 4). Direct conjugation of oxcarbazepine, in the enol form, is a minor pathway. Oxidative reactions are thought to be unimportant. Following administration of single oral doses of 400 mg of [^{14}C]-labeled OXC to two healthy volunteers, 2.0% of total plasma radioactivity (0-72 h) was attributable to unchanged OXC and 68.4% to MHD. DHD (inactive metabolite) accounted for 6.4%. On average, 99% of the dose was excreted in urine and feces within 10 days. Renal excretion was predominant, amounting to 95.5% of the dose. MHD accounted for 71% of total urinary radioactivity, 28% unchanged and 43% as the glucuronide conjugate (**Table IID.2**). OXC (unchanged and conjugated) and DHD accounted for about 14% and 4% of urinary radioactivity, respectively. Trace amounts (< 0.6%) of the cis-diol metabolite (a conformational isomer of DHD) and an unidentified metabolite with a hydroxyl group in position 2, 3, 7 or 8 of the aromatic moiety were also identified.

GP 47779 (MHD)

Oxidation of MHD to OXC and DHD was much less extensive in humans than in rats or dogs. Following single oral doses of 400 mg of [^{14}C]-labeled racemic MHD in two healthy volunteers, 64.7% of the total plasma radioactivity was accounted for by MHD, 4.9% by DHD and 1.2% by OXC, formed as a metabolite (**Table IID.2**). The recovery of total radioactivity in urine and feces was almost complete within 7 days. Parent MHD, OXC, and DHD represented about 93% of the total radioactivity in urine, with MHD accounting for a much higher percentage of total radioactivity (83% after enzyme trmt) than the two metabolites (OXC:3%; DHD:7%).

Enzyme Induction/Inhibition

Dose-dependent induction of liver microsomal cytochrome P450 (cytochrome P450-associated monooxygenases, microsomal epoxide hydrolase, UDP-glucuronosyltransferase, and cytosolic glutathione S-transferase) was demonstrated in rats following repeated administration of OXC, MHD, or carbamazepine (10, 50, 100, and 200 mg/kg). T-R changes in the microsomal content of individual cytochrome P450 isoenzyme proteins included pronounced increases in the levels of CYP2B1 and CYP2B2 and moderately increased CYP2C6 levels. Auto-induction was also demonstrated when the same doses of these 3 compounds were administered to rats in a toxicokinetic study. After repeated dosing with 200 mg/kg (12 days; po), plasma AUC values for carbamazepine, OXC and MHD were 40.5%, 21.6% and 67.2%, respectively, of the corresponding values obtained after a single dose (**Tables IIB.2** and **IIB.4**). Because equivalent doses did not result in equivalent initial plasma levels of the administered compound (ie, OXC > MHD), it is not clear that this is a valid comparison. However, the relationship appeared to hold when more comparable exposures were compared in this study. Total exposures to pharmacologically active compounds (OXC + MHD) were similar when the same dose of either was administered, but decreased less after repeated administration of MHD than after OXC, which might account for some differences in long-term toxicity between the 2 compounds. A more recent study showed a 60% reduction in MHD AUCs following po administration of 100 mg/kg MHD to rats for 14 days. In this study, OXC AUCs increased over the same period, indicating induction of the oxidative pathway by which MHD is metabolized to OXC. When the enzyme induction was studied *in vitro* in cultured human hepatocytes, UDP-glucuronyltransferase activity was increased 22%, 47% and 39% by MHD, OXC and carbamazepine, respectively, at a concentration of 200 uM. In another *in vitro* study using human hepatocytes (not submitted), P-450 isozymes of the 2B and 3A families were reportedly induced by both OXC and MHD, but MHD was said to be less potent than OXC. Both OXC and MHD were found to be

weak inhibitors of human cytochrome CYP3A4/5 and CYP2C19 *in vitro*. Based on the Ki values (647 and 88 uM, respectively, for MHD), only the effect on the latter was thought to be clinically relevant.

Toxicokinetics

Plasma level data from repeated-dose studies in mice, rats, and dogs and human exposure data are summarized in **Table IIF.1**. The doses for which human data are available are lower than the maximum anticipated clinical dose of 2400 mg. In studies in which OXC or MHD was administered to rats and dogs, systemic exposures to OXC were higher but exposures to MHD were much lower than those measured in humans. Unlike the humans situation, reduction of OXC to MHD is not an important route of metabolism in rats and dogs, so that unchanged drug was the predominant active component in plasma after administration of OXC to these species. When MHD is administered directly to rats, much of it is back-oxidized to OXC, resulting in OXC/MHD AUC ratios of about 1. Oxidation of MHD also occurs in the dog, although to a lesser extent than in rats. When dogs were administered MHD, the AUC ratios were between 0.1 and 0.2 (see **Table IIB.6**). OXC/MHD AUC ratios were approximately 0.03 and 0.02, respectively, after administration of OXC and MHD to humans. This would appear to make the dog administered MHD the more relevant of the two preclinical models used; however, toxicokinetics and metabolism were poorly characterized in dogs. The lack of TK data for repeated-dose administration of MHD to dogs is a major deficiency

TOXICOLOGY

Repeated Dose - Rat

GP 47680 (OXC)

Repeated oral dose toxicity studies of GP 47680 were conducted in rats for 3 months (100, 300, 1000, and 3000 mg/kg by gavage) and 6 months (100, 300, and 1000 mg/kg in the diet) using synthesis 1 drug and for 3 months (100, 300, 1000, and 3000 mg/kg by gavage; 20, 60, and 300 mg/kg by gavage) and 6 months (10, 45, or 150 mg/kg) with synthesis 2 material. Findings in these studies included mortality at 3000 mg/kg in males and at ≥1000 mg/kg in females, CNS-related clinical signs (ataxia, hypoactivity, ptosis, tremors) and decreased BW gain at ≥1000 mg/kg in males and ≥300 mg/kg in females, clinical pathology changes indicative of increased RBC turnover (↑ MCV, MCH, reticulocytes, echinocytosis) and altered hepatobiliary (↑ GGT, cholesterol, bilirubin, bile acids, ALP, ALT) and renal tubular (↑ BUN, polyuria, proteinuria) function at ≥100 mg/kg, increased liver weights and hepatocellular hypertrophy at ≥45 mg/kg in females and ≥60 mg/kg in males, hepatocyte vacuolar degeneration and necrosis at ≥300 mg/kg, increased hepatocytic mitotic figures in female decedents at ≥1000 mg/kg, and nephropathy in males at ≥100 mg/kg and females at ≥300 mg/kg (see **Tables IIIA.1** and **IIIB.1**). T-R microscopic findings in the liver and kidneys were also seen in the 2-year rat carcinogenicity study of GP 47680 (synthesis 1; ≥25 mg/kg), where incidences of liver cell adenoma (males) and carcinoma (females) were increased (**Table IVB.4**). It should be noted, that effects were observed at lower doses and/or were of greater severity in the 13-week study using GP 47680 produced with the current synthesis method, i.e., synthesis 2, than in the earlier 13-week study of synthesis 1 GP 47680 in rats. Although some additional effects were also reported in the more recent (synthesis 2) study, this could simply reflect the thoroughness of the evaluation. The new synthesis method introduced low levels of several impurities which had not been present in drug produced by the original method, which could result in intrinsic differences between synthesis 1 and 2 drug. A more likely explanation for the apparent difference in potency was that the change in synthetic route was accompanied by a reduction in drug substance particle size and better control of particle size distribution, which could have increased bioavailability. However, an exposure comparison between synthesis 1 and 2 was not performed in either rats or dogs.

The elevations in MCV, MCH, reticulocyte counts, and (possibly) serum bilirubin point to increased red cell destruction and were thought to be directly related to T-R echinocytosis, for which there was no explanation. The urinalysis changes were thought to reflect the renal tubular damage observed histologically. Renal functional effects were also demonstrated in safety pharmacology studies, where increases in urine volume and electrolyte excretion and, in some cases, decreased plasma sodium levels were observed in rats given

single oral doses (100-300 mg/kg) of OXC or MHD (or CBZ). Because this effect was diminished or absent in Brattleboro rats, which lack antidiuretic hormone, an ADH-related mechanism was indicated. However, hyponatremia was not seen in the repeated-dose studies of OXC. The increases in gamma-GT, cholesterol, ALP, and bilirubin indicate cholestasis, presumably due to obstruction or hepatopathy, and there was evidence of hepatocellular damage in addition to typical adaptive changes associated with enzyme induction.

Plasma drug levels determined during these studies (**Tables IIIA.2** and **IIIB.2**) indicate that autoinduction occurs and that females were exposed to considerably higher concentrations of OXC and MHD than males. These results would explain the the observed tolerance to the CNS effects and the increased incidence and/or severity of some effects in females. Although plasma levels of OXC at doses associated with toxicity in the chronic rat studies (≥100 mg/kg) were greater than those expected clinically, rat exposures to MHD, in the study in which it was measured, and total exposures (OXC + MHD) were much lower than human clinical exposures (see **Table IIF.1**).

GP 47779 (MHD)

Repeated oral dose toxicity studies were conducted in rats for 3 months (200, 600, or 2000 mg/kg by gavage) and 6 months (approximately 50, 200, or 600 mg/kg in the diet) using synthesis 1 GP 47779 and for 13 weeks (50, 200, 600, or 2000 by gavage) with synthesis 2 GP 47779. Findings were qualitatively similar to those reported for OXC and included mortality at 2000 mg/kg, clinical signs of CNS toxicity (ataxia, hypoactivity) at ≥600 mg/kg, decreased BW parameters at ≥600 mg/kg, hematologic changes (↑ MCV, MCH, echinocytes; ↓ WBCs) primarily at ≥600, clinical pathology changes (↑ GGT, cholesterol, bilirubin, bile acids, ALT, ALP) primarily at ≥200 mg/kg, polyuria and proteinuria at ≥50 mg/kg, increased liver weights at ≥50 mg/kg and hepatocellular hypertrophy (with intracytoplasmic inclusion bodies) at ≥200 mg/kg (marked at 2000 mg/kg), hepatocyte necrosis at ≥600 mg/kg, and brown pigment in hepatocytes at 2000 mg/kg. Effects on the kidney were not reported in studies with synthesis 1 MHD, but minimal nephropathy (hyaline casts & tubular dilatation) was observed in males at ≥600 mg/kg in the study of synthesis 2 MHD (**Table IIIC.2**). In the 2-year rat carcinogenicity study of MHD (Synthesis 2: 75, 250, or 600 mg/kg by gavage), increased incidences and/or severity of renal lesions were seen in both sexes at ≥75 mg/kg (**Table IVC.4**). These included typical chronic nephropathy as well as less common findings such as thrombosis of large caliber hilar veins in males and (golden brown granular) pigmentation of the renal tubular epithelium in females. Incidences of a variety of non-neoplastic liver alterations, including biliary lesions, were also increased in treated rats at all dose levels in this study, in which hepatocellular adenoma and carcinoma were also increased (**Table IVC.5**). Most of the findings reported with synthesis 2 GP 47779 had not been observed or had been seen only at higher doses in the studies performed with synthesis 1 material. This was thought to reflect the quality of the studies as well as differences in potency/bioavailability (see above) of the drug.

Toxicokinetic data (**Table IIIC.3**) showed that significant amounts of OXC were formed from the administered MHD, such that plasma levels of each were similar for a given sex. This and the different doses used in the OXC studies make it difficult to assess the relative toxicities of the two compounds in rats. However, it appeared that qualitatively and quantitatively similar effects were seen at comparable doses in studies in which MHD was administered (exposure to OXC and MHD) as compared to those in which OXC was given (exposure primarily to OXC), indicating approximately equivalent toxic profiles and potencies (see MHD carcinogenicity in rats below). Although levels of MHD as well as OXC and DHD were considerably higher in females than in males at the same dose, presumably due to higher clearance in males, and effects were often seen at lower doses or with greater incidence and severity for a given dose in females; there was not always a clear correlation between plasma concentrations and toxicity, suggesting the possibility of an unidentified toxic metabolite(s) or of some other relationship between metabolism and toxicity. Although plasma levels of OXC at doses associated with toxicity in the chronic rat studies (≥50 mg/kg) were greater than those expected clinically, rat exposures to MHD and total exposures (OXC + MHD) were considerably lower than human clinical exposures (**Table IIF.1**).

Multicose iv studies were conducted with GP 47779 (synthesis 2) in rats (5, 12.5, or 25 mg/kg for 14 days; 1.5, 4.5, or 12.5 mg/kg for 3 months), but because of the low doses used, no significant effects were observed

(reviewed under IND 54,554 for iv formulation of GP 47779). The exposures achieved by iv administration (mean AUCs of 13 and 22 ug.h/ml for GP 47779 in M and F rats, respectively, at 12.5 mg/kg iv) were much lower than expected human exposures. The HD in the 13-week study was said to be the "technically maximum applicable dosage, based on a dosage volume of 5 ml/kg and a concentration of 2.5 mg/ml," which is the concentration of the clinical formulation.

Repeated-Dose - Dog

GP 47680 (OXC)

In repeated-dose oral (capsule) toxicity studies of OXC in dogs (synthesis 1: 60, 200, and 600 mg/kg for 3-months; 60, 200, and 600-400 mg/kg for 12 months; synthesis 2: 100, 300, and 600 mg/kg for 2 weeks; 200, 400-800, and 600 mg/kg for 4 weeks; 60, 200, and 600 for 12 months), relatively mild effects were reported at the doses examined. It appeared that acute neurotoxicity and vomiting were the main dose-limiting factors. In addition, the drug was poorly absorbed in dogs, and feces containing the drug were seen at all doses. Effects included CNS signs (ataxia, loss of righting reflex, recumbency, clonic convulsions) and emesis at ≥400 mg/kg, decreased BW gain at 800 mg/kg, slight decreases in RBCs (and hemosiderin deposits in liver and kidney) and changes in coagulation parameters (APTT and PTT prolonged) at ≥200 mg/kg, clinical chemistry alterations (↑ cholesterol, triglyceride, and bile acids; ↓ phosphorus) at ≥60 mg/kg, increased liver enzymes (ALT, AST, and ALP) at ≥600 mg/kg, and increased liver weights and hepatocellular cytoplasmic vacuolization at ≥60 mg/kg. No renal toxicity was observed in dogs treated with OXC. Despite evidence of melanin binding in the tissue distribution study, there were no T-R ophthalmological changes. Findings were generally consistent across studies, and similar toxicity profiles were observed in studies conducted with synthesis 1 and synthesis 2 material, although additional findings were reported in the more recent studies. In particular, decreased HR (2/3 HD M) and second degree AV block (1/3 HD M) were recorded (4 hr after dosing on day 11) in dogs receiving 600 mg/kg of 'extrafine' synthesis 2d GP 47680 in a very recently conducted ('98) 2-week study. Cardiovascular effects had not been reported in any of the previous toxicology studies of OXC, either with synthesis 1 or 2 material. In an early safety pharmacology study, OXC administration exacerbated what were thought to be pre-existing ECG abnormalities (fluctuations in P wave amplitude and ventricular extrasystoles were seen predosing) in one dog. The 2-week study was the only toxicology study in which 'extrafine' drug that had been milled to further reduce particle size was used. The significance of the CV effects reported in this study is not clear. The sponsor stated that "since there were no relevant changes in electrocardiography in the previous toxicity studies with GP 47680, an incidental nature of this finding seems likely; a possible relationship to treatment, however, cannot be excluded entirely." In a safety study that evaluated the CV effects of MHD enantiomers, it was suggested that the ECG alterations seen (below) could have resulted from drug-induced changes in electrolyte levels. It is also possible that they resulted directly from the actions of these compounds on ion channels. When the potential of GP 47779 to produce hemolysis was tested in vitro using heparinized blood from beagle dogs, no hemolysis was seen with an aqueous solution of 50 mg GP 47779/20 ml.

There are no good toxicokinetic data in dogs administered OXC, reportedly due to the instability of OXC in dog plasma. Approximate Cmax values from the 2-week study (**Table IIIF.1**) indicate that relatively high but variable peak levels of OXC were achieved (>10 ug/ml at 600 mg/kg), while levels of MHD were very low (<0.5 ug/ml). There was no consistent evidence of time- or sex-dependent kinetics based on this limited data. Plasma level data in male dogs administered single oral doses of OXC (synthesis 1: 5, 30, 300 mg/kg) are shown in **Table IIB.5**.

GP 47779 (MHD)

Repeated oral dose toxicity studies of synthesis 1 GP 47779 were conducted in dogs for 3 months (60, 200, and 600-400 mg/kg) and 1 year (30, 100, and 300-200 mg/kg). No oral studies have been conducted in dogs with synthesis 2 GP 47779. In both cases, the study director lowered the HD because of excessive toxicity (clinical signs, BW loss), indicating that the MTD was exceeded. Although there were 3 deaths in the 1-year study (1 in each dose group), these were not considered T-R. Other findings included clinical signs of CNS

toxicity (ataxia, hypoactivity, depression, prostration, tremor, opisthotonos) at doses ≥30 mg/kg, decreased BW gain at ≥200 mg/kg, evidence of hematological toxicity (↓ erythrocyte parameters - sometimes marked, ↑ or ↓ reticulocytes, hemosiderin deposition, extramedullary hematopoiesis) at ≥100 mg/kg, clinical chemistry changes indicating effects on the liver (↑ ALP) at ≥100 mg/kg and on electrolyte balance (↑ sodium, ↓ potassium) at ≥30 mg/kg, increased liver weights at ≥30 mg/kg and decreased heart weights at ≥400 mg/kg, and microscopic pathology of the liver (hepatocellular bile accumulation, inflammation, hepatocyte necrosis) at ≥200 mg/kg and kidney (pigment granules and vacuoles in the proximal convoluted tubules) at ≥400 mg/kg. In addition, a significant increase in mean P-R interval in was seen in female dogs at 300-200 mg/kg in the 1-year study. Increases in P-wave amplitude and duration and D-D increases in the P-R interval were seen in dogs after single iv or oral doses of MHD or its enantiomers in safety pharmacology studies. P-R interval prolongation and second degree AV block were also seen in a 2-week study of OXC in dogs, at an oral dose of 600 mg/kg. Although tissue distribution studies of MHD indicated melanin binding in dogs (pigmented rats not examined), there was no clear evidence of T-R ophthalmologic changes. While most of the findings with MHD were qualitatively similar to the effects of OXC in dogs (as well as the results in rats), they were sometimes more severe (hematologic effects) or were seen at lower doses (clinical signs). The apparently greater toxic potency of MHD compared to OXC may have been due to better absorption, but no kinetic data were collected in the toxicity studies of MHD in dogs. Plasma levels measured in male dogs administered single oral doses of MHD (synthesis 1: 5, 30, 150 mg/kg; **Table IIB.6**) suggest that MHD exposures approaching those expected in humans could have been achieved at the doses used in the toxicity studies (see **Table IIF.1**); however, the effect of repeated dosing on plasma levels in dogs is unknown. In addition, these early plasma level determinations may be unreliable, based on comparisons of rat data from the same time with more recent measurements in rats.

Multidose iv studies were conducted with GP 47779 (synthesis 2) in dogs (3 or 10 mg/kg for 14 days; 2, 6, or 12.5 mg/kg for 13 weeks), but because of the low doses used, no significant effects were observed (reviewed under IND 54,554). The exposures achieved by iv administration (mean AUCs <20 ug.h/ml at 12.5 mg/kg iv in dogs) were much lower than expected human exposures. The HD was said to be the "technically maximum applicable dosage, based on a dosage volume of 5 ml/kg and a concentration of 2.5 mg/ml," which is the concentration of the clinical formulation.

CARCINOGENICITY

Mouse - *GP 47680 (OXC)*

The mouse carcinogenicity study of OXC (synthesis 2) was conducted with doses of 0, 10, 40, 70, and 100 mg/kg administered in the diet for 2 years. There was an apparent treatment-related effect on survival in HD males: during weeks 71-74, 18 HD males died compared to 4 C; and 10/18 HD males that died were found to have myocardial necrosis and hemorrhage when examined histopathologically (**Table IVA.1**). There were no differences in survival rates thereafter, and such lesions were not found in any males that died or were sacrificed after week 74. Comparable effects on the heart were not seen in toxicology studies in rats or dogs, so the significance of these pathological changes, which are fairly nonspecific, is difficult to assess without additional information in this species. Localized myocardial necrosis is a frequent finding in aged mice, and a variety of factors, such as disturbances in oxygen supply or ionic balance, can increase its incidence. Despite this effect, an adequate number of HD males survived to termination. BW gain was reduced slightly in MHD and HD males compared to C during the treatment period, but there were no group differences in BWs at the end of the study. BW gain was decreased somewhat in all female treatment groups compared to C, and at termination mean BWs in MD, MHD, and HD females were about 10% below C (SS at MD and HD). Liver enzymes (ALT, AST, and ALP) were generally elevated in treated males and females at all does compared to C, and liver weights were increased in both sexes at the 3 highest doses. Upon gross examination, enlarged livers and increased numbers of liver masses were observed in treated males (all doses) and females (top 2 doses). Non-neoplastic histopathology findings included the myocardial hemorrhage and necrosis in HD males (also seen in 1 MHD male) and low incidences of liver fibrosis and nodular hyperplasia in HD males. The only apparent T-R neoplastic finding was an increased incidence of benign hepatomas in treated (MHD and HD) males and (HD) females (**Table IVA.2**). Only the HD male effect

was significant in the FDA statistical analysis. Very limited plasma level data were collected (1 time point at 6, 12, 18, and 24 months); these indicated that OXC concentrations were low (~0.6 ug//ml at HD) but similar between sexes. MHD levels were usually below the limit of detection.

Rat

GP 47680 (OXC)

The rat carcinogenicity study of OXC (synthesis 1) was conducted with doses of 0, 25, 75, and 250 mg/kg administered in the diet for 2 years. There were no significant T-R differences in mortality rates, clinical signs, or clinical pathology parameters; however, BW gain and food consumption were reduced by treatment, and terminal BWs were significantly lower in HD males (15%) and in MD (15%) and HD (26%) females compared to C. When ophthalmological examinations were performed at 2 years, several ocular lesions were increased in incidence in treated rats; these included lenticular suture cataracts, capsular cataracts, and keratitis. Kidney and liver weights were increased in MD and HD males and females, but there were no gross findings. A variety of non-neoplastic microscopic liver and kidney changes were found; these included hepatocellular hypertrophy and vacuolar and cystic degeneration of hepatocytes and chronic progressive nephropathy and various associated renal lesions in males and females from all treatment groups. There were apparent T-R increases in the incidences of hepatocellular adenomas (termed neoplastic nodules in report) in males and hepatocellular carcinomas in females (**Table IVB.4**). In addition, incidences of Leydig cell tumors were increased in treated males. In the FDA statistical analysis, the effect on interstitial cell tumors was significant at the HD in males, while the increase in hepatocellular carcinomas approached but did not reach significance in HD females. No toxicokinetic data were collected in this study.

Increased incidences of neoplastic liver lesions were also observed in a 2-years rat study of carbamazepine(CBZ), but different diagnostic terms were used for the lesions in the 2 studies, which were conducted by different labs (CBZ by [redacted]/OXC by [redacted]) Therefore, 2 pathologist from C-G reexamined the slides from these studies in order to compare the hepatocellular lesions. It was the opinion of these pathologists that the lesions referred to in the CBZ study as hepatomas (incidences: 5/60, 2/60, 3/60, and 6/60 in males; 2/60, 12/60, 12/60, and 27/60 in females at 0, 25, 75, and 250 mg/kg, respectively) and in OXC study as either benign neoplastic nodules (5/60, 7/60, 8/60, and 9/60 in males; 2/60, 7/60, 6/60, and 3/60 in females at 0, 25, 75, and 250 mg/kg, respectively) or malignant hepatocellular carcinomas (2, 1, 6, and 2 in males; 0, 3, 7, and 7 in females) were very similar histologically, and it was proposed that the term liver cell tumor be used for all of them. The distinction between benign neoplastic nodule and malignant hepatocellular carcinoma was considered to be inexact. Furthermore, it was determined that the tumors in both studies showed no signs of invasiveness or metastasis and did not influence survival.

The ability of oxcarbazepine to promote the formation of focal proliferative changes in the rat liver was also examined in a neonatal rat model. 24 hr after birth, rat pups received an ip injection of either N-nitrosodiethylamine or vehicle, then at 3 weeks were assigned to treatment groups to receive either GP 47680 (2000 ppm in diet), phenobarbitol (positive control; 500 ppm), or control diet for up to 8 weeks. Animals were then sacrificed and livers were examined for proliferative changes by staining for gamma-glutamyl-transpeptidase (GGT) activity. The development of GGT-positive foci was enhanced to a comparable degree by both phenobarbital and GP 47680. It was concluded that with respect to the foci enhancing or promoting effects, exposure to 2000 ppm GP 47680 was similar to exposure to 500 ppm phenobarbital.

GP 47779 (MHD)

In the rat study of MHD (synthesis 2), doses of 0, 75, 250, and 600 mg/kg were administered by gavage for 2 years. Survival was increased in HD males and females compared to C. Clinical signs (ataxia, hypoactivity) were noted in HD males and females, and BW gain and final BWs were significantly reduced in HD males (15%) and MD (12%) and HD (36%) females, indicating that adequate doses were used. No hematological or ophthalmological changes were observed (clinical chemistry not performed). Numbers of animals with grossly observable hepatic nodules (reported as tissue masses) and lesions and incidences of a variety of

non-proliferative (hepatocellular hypertrophy, biliary cysts, cystic degeneration, periportal fibrosis, vacuolation, necrosis) and proliferative (foci of cellular alteration, hyperplasia, hepatocellular adenoma, carcinoma) microscopic liver changes were increased at all doses in both sexes (**Tables IVC.3** and **IVC.5**). Evidence of T-R kidney changes (chronic nephropathy, thrombosis of hilar veins, granular pigmentation of tubular epithelium) was also seen at all doses (**Tables IVC.4**). Other neoplastic findings consisted of increased incidences of Leydig cell tumors in MD and HD males and granular cell tumors of the cervix and/or vagina in females at all doses (**Tables IVC.6- 7**). Thyroid follicular hyperplasia/hypertrophy was also increased in treated males and females. In the FDA statistical analysis, the increase in hepatocellular adenoma was significant in HD males, and incidences of hepatocellular adenoma and carcinoma were each significantly increased in HD females; however, the increased rates of genital organ tumors failed to reach significance. Plasma levels of MHD and DHD (CGP 10000) were measured only at 2 hr after dosing during weeks 1 and 52 (**Table IVC.8**). MHD levels at this one time point were consistently higher in females and generally increased over the course of the study in both sexes. OXC levels were not determined.

Possible differences in the spectrum of neoplastic and nonneoplastic lesions and their incidences and severities between the 2-year studies of OXC and MHD are difficult to assess in light of the differences in study conduct, doses, and routes. No plasma level data were collected in the 2-year OXC study, so exposure comparisons cannot be made. Overall, though, it appears that the findings were comparable. For example, incidences of chronic progressive nephropathy and hepatocellular hypertrophy were increased at all doses in both studies; hepatocellular carcinoma occurred in females at ≥25 mg/kg OXC and ≥250 mg/kg MHD, but the incidence at a dose of 250 mg/kg was 7/60 for OXC and 6/60 for MHD; biliary cysts were seen in a total of 8/60 females treated with 250 mg/kg OXC, and the same incidence was reported at the same dose of MHD; and incidences of Leydig cell tumors were almost identical in the two studies at a dose of 250 mg/kg. Although granular cell tumors of the cervix/vagina were not reported in the OXC study, vaginal samples were not collected and, according to the sponsor, "collection of the cervix with the uterus was not standardized at the time the study was performed." The non-genotoxic mechanisms proposed for the 3 tumors seen in this study - liver tumors were attributed to increases in altered cell foci secondary to the induction of microsomal enzymes and/or increased cell proliferation due to hepatotoxicity and cell death; Leydig cell and granular cell tumors were attributed to perturbations in hormone homeostasis (LH and estrogen, respectively), possibly secondary to enzyme induction - are plausible, but have not been adequately substantiated to include in labeling.

GENOTOXICITY

GP 47680 (OXC)

In the Ames test, OXC (synthesis 2) produced a dose-dependent increase in revertant numbers in strain TA 100, without activation (**Table VA**). An earlier study with synthesis 1 OXC and a follow-up Ames test with synthesis 2 were both negative, however. There was no good explanation for the difference in results between the three studies. In the positive study, similar increases in revertant numbers were seen with batches of synthesis 2 OXC containing the impurities normally present and with a highly purified batch of OXC, so the mutagenic effect was not thought to be due to impurities. No effect on mutation frequencies was seen in a forward mutation (HGPRT locus) assay in cultured V79 Chinese hamster lung cells. In a CHO clastogenicity test, chromosomal aberrations and polyploidy were increased by OXC in an experiment conducted with a 42 hr treatment time without activation (**Table VC**). The clastogenic effect seen in CHO cells was thought to be related to the occurrence of polyploidy. Polyploidy was also increased in a V79 Chinese hamster cell clastogenicity assay without a clear increase in chromosomal aberrations. OXC (synthesis 2) was negative for clastogenic and aneugenic effects (micronucleus formation) in an *in vivo* rat bone marrow assay.

GP 47779 (MHD)

No evidence of mutagenicity was observed with MHD (synthesis 2) in the Ames test. When the ability of MHD to induce mutations at the HGPRT locus was evaluated in CHO and V79 cells, there were no increases in mutation frequencies at concentrations of up to 2 mg/kg; however, in the non-GLP CHO study, increased mutant frequencies were seen at a cytotoxic concentration of 4 mg/ml, with and without metabolic activation

(**Table VH**). In a CHO chromosomal aberration test, percentages of cells with chromosomal aberrations were concentration-dependently increased compared to the negative control under some exposure conditions (18 hr treatment period without metabolic activation; **Table VJ**), and an increase in numerical chromosomal aberrations (polyploidy) was seen at the high concentration (1000 ug/ml). No increase in micronucleus formation was seen at MHD doses of up to 2960 mg/kg in an *in vivo* rat bone marrow assay.

REPRODUCTIVE TOXICOLOGY

Fertility and Reproductive Performance

GP 47680 (OXC)

An early non-GLP Segment I study of synthesis 1 GP 47680 was conducted in rats with oral doses of 25, 50, and 150 mg/kg administered to males and females prior to and during mating and gestation and throughout lactation. There appeared to be a small effect on weight gain in females (D-R decrease shown graphically), but this was not quantified. No toxicity was observed in adult males. There were no clear effects on fertility. At term sacrifice, resorptions were slightly increased in HD litters and fetal weights were slightly but D-D decreased in MD and HD litters (**Table VIA.1**). Corpora lutea were not counted. No increase in abnormalities was reported, but fetuses were only examined externally. Pup weights appeared to be decreased slightly in MD and HD groups through weaning (data only provided graphically; **Figure VIA.2**). There were said to be no group differences in the attainment of physical and functional developmental landmarks, but the data were not provided. Exploratory behavior expressed as an activity index was slightly reduced in HD offspring on PND 32. Learning and memory were not assessed in the offspring. This study was inadequate both in conduct and reporting, but the more relevant studies of MHD in rats covering the same endpoints (fertility and early development and pre- and postnatal development) were adequate (below).

GP 47779 (MHD)

A fertility and early embryonic development study (ICH design) of synthesis 2 GP 47779 was conducted in rats with oral doses of 50, 150, 450 mg/kg given to males and females prior to and during mating through GD 6. Dose selection was appropriate, based on BW effects in males (BW gain over the course of the study 25% below C) and females (gestational BW gain, corrected GD 13 BW reduced; **Table VIB.1**). While mating and fertility parameters were comparable among groups, the number of acyclic females was increased and the number with regular cycles reduced in the HD group during the premating period. Numbers of corpora lutea, implants, and live embryos were reduced in treated females compared to controls, reaching statistical significance at the HD (**Table VIB.2**). There were no apparent T-R differences in pre- or postimplantation loss. Testes weights were not affected by treatment, and while sperm analysis suggested possible slight effects on sperm numbers and motility, there were no statistically significant differences among groups for these parameters (**Table VIB.3**).

Embryo-Fetal Development

GP 47680 (OXC)

Mouse

In an early non-GLP mouse Segment II study of synthesis 1 GP 47680 (25, 75, or 250 mg/kg by gavage on GDs 6 to 15), the only effects reported were maternal clinical signs and a small decrease in fetal weights at the HD (**Table VIC.1**). This study was clearly inadequate, but adequate studies are available in rats and rabbits (below).

Rat

A rat Segment II study of synthesis 2 GP 47680 was conducted with doses of 30, 300, or 1000 mg/kg given

by oral gavage on GDs 6 to 15 (carried out in '94; does not conform to ICH). The HD was clearly maternally toxic as evidenced by decreased BW gain (**Table VID.1**), clinical signs, and 1 death. Clinical signs and a transient BW effect were also seen at the MD, which appeared to be closer to an MTD. Resorptions were increased (**Table VID.2**) and fetal body weights were markedly decreased in HD litters. Fetal and litter incidences of external (2/252, 2/19), visceral (4/78, 4/19) and skeletal (3/174, 3/19) malformations were increased at the HD (all SS) compared to C (no malformations found in C or LD; **Table VID.3**). Incidences of external (1/366, 1/24) and visceral (5/116, 3/24) malformations were also increased at the MD. Defects included various craniofacial malformations (eg, cleft palate, microphthalmia), heart malformations (septal defects, persistent truncus ateriosus) and agenesis, fused, and/or misaligned vertebrae. Although maternal toxicity could be considered excessive at the HD, where most of the malformations occurred, individual data did not indicate any correlation between maternal toxicity and malformations. Incidences of visceral (lung and liver irregularities) and skeletal (cervical rib) variations were also increased and ossification of various skeletal elements was reduced in MD and HD litters.

GP 47779 (MHD)

Rat

In a non-GLP oral rat Segment II study of Synthesis 1 GP 47779 (75, 250 and 500 mg/kg on GDs 6-15), maternal toxicity (decreased BW gain, deaths) and small non-D-R increases in resorptions were seen at the MD and HD. This study, like the others from this period, is of poor quality, and so the results must be viewed with caution. In the fetal evaluations, for example, no malformations of any kind were found in any group.

Rabbit

A Segment II study (conducted in '94) of synthesis 2 GP 47779 was conducted in rabbits with oral (gavage) doses of 20, 100, or 200 mg/kg administered on GDs 7-19. Clinical signs and slightly decreased BW gain during the treatment period were observed in HD does (**Table VIE.1**). Corrected GD 29 BWs and BW gains were not different among groups, however. In a dose range-finding study, convulsions were seen at ≥500 mg/kg, but minimal maternal toxicity occurred at 250 mg/kg; so dose selection for this study is questionable based on maternal effects, but in view of increased resorption at the HD is probably appropriate. Resorptions (early and late) were increased in all treatment groups (total SS at all doses compared to C; **Table VIE.3**). Percent postimplantation loss was 10-fold C at the HD, but the LD and MD effects were small and not D-R and did not result in a decrease in live fetuses. Fetal body weights were decreased slightly in MD and HD females (both 3% below C; NS). There were no apparent effects on fetal structure; incidences of malformations and variations were similar among groups (**Table VIE.3**). It should be noted that because of increased resorption fewer fetuses were evaluated at the HD compared to other groups. However, the number of litters evaluated was probably adequate. The highest dose tested in this study produced relatively high MHD exposures compared to those measured in rats during the chronic toxicity studies (**Table VIE.2**).

Pre- and Postnatal Development

GP 47680 (OXC)

A non-GLP oral Segment III study of synthesis 1 GP 47680 was conducted in rats with doses of 25, 50, or 150 mg/kg administered from day 15 of gestation through weaning. Maternal BW gain appeared to be decreased (D-R decrease shown graphically), but this was not quantified. There were 2 HD dams deaths (days 20 and 22, both with dead fetuses) due to apparent dystocia, and an additional 1 HD and 3 MD dams suffered total litter loss. Not counting these losses, mean litter size at birth was decreased at the HD, but there was no apparent effect on postnatal survival. Pup BWs were decreased in HD litters through day 35. There were said to be no group differences in the attainment of physical and functional developmental landmarks, but the data were not provided. Learning/memory and reproductive function were not assessed in the offspring, but exploratory behavior expressed as an activity index was reduced in HD offspring during testing between PNDs 29-32. This effect was seen at the same dose in the Segment I study of GP 47680.

GP 47779 (MHD)

A GLP pre-/postnatal study of synthesis 2 GP 47779 was carried out in rats with doses of 25, 75, or 250 mg/kg given by oral gavage from GD 6 through PND 20. Adequate maternal toxicity was seen at the HD (clinical signs, decreased BW gain). There was an unexplained reduction in the numbers of pregnant dams evaluated at the HD; although there were 25 assumed pregnant per group initially, only 19 HD dams were evaluated. Gestation length was increased in the HD group, but other reproductive parameters (postimplantation loss, viable newborn) were similar among groups. Litter sizes and pup survival indices were comparable between treated and control groups, but pup weights were decreased in HD litters and this deficit persisted into the postweaning period (**Table VIE.3**). There were no apparent T-R effects on the attainment of pre- and postweaning developmental landmarks, and no SS group differences in the limited behavioral evaluations performed on F1 animals (activity and passive avoidance). However, female pups showed small but D-R increases on two measures of activity in the open field test. F1 fertility was not affected by treatment.

CONCLUSIONS

Oxcarbazepine (OXC) is a 10-keto-derivative of the widely used antiepileptic drug carbamazepine (CBZ). In contrast to the primarily oxidative metabolism of CBZ, the major metabolic pathway for OXC in humans involves reduction to an active monohydroxy derivative (MHD), without epoxide formation (**Figure VIII.1**, below). It was anticipated that this difference in metabolism would confer a safety advantage on OXC, since the epoxide has been implicated in the some of the toxic effects of CBZ (eg, its teratogenicity). And because of the limited involvement of oxidative microsomal enzymes in its metabolism, it was thought that OXC would have less capacity for enzyme induction and drug interactions than CBZ. Pharmacology results indicate that OXC and CBZ have comparable *in vivo* anticonvulsant profiles and potencies; and the mechanism of action of OXC, like that of CBZ, is thought to involve effects on voltage-dependent sodium channels. In humans, OXC is rapidly and almost completely converted to MHD, which is present in plasma at levels much greater than (10-60X) those of the parent and is thought to be responsible for most of the drug's antiepileptic activity (**Figure VIII.2**). Thus, OXC is considered to be a pro-drug for MHD. Anticonvulsant testing in animals indicates that MHD and OXC have similar efficacy under both *in vivo* and *in vitro* conditions. Equivalent efficacy of OXC and racemic MHD is important, because while conversion of OXC to MHD is virtually complete in humans, it is much less pronounced in other species. (The enantiomers of MHD also seem to have approximately equivalent anticonvulsant efficacy).

None of the animal test species investigated had a metabolic profile qualitatively and quantitatively similar to that of humans, with the possible exception of the baboon, which was only examined preliminarily. Reduction to MHD appears to be only a minor pathway for the metabolism of OXC in mice, rats, and dogs; and in contrast to the situation in humans, unchanged drug was the predominant component in plasma following administration of OXC in these species. This is clearly problematic for the preclinical safety evaluation. A proposed solution was to conduct much of the preclinical toxicity testing with the metabolite, MHD. But here again the experimental animals were different from humans: both rats and dogs back-oxidize MHD to OXC to a much greater extent than humans. When MHD was administered to rats, systemic exposures to OXC and MHD were similar. Although oxidation of MHD to OXC was less extensive in dogs - the OXC/MHD plasma AUC ratio was between 0.1 and 0.2 following single dose administration of MHD to dogs - it was still more pronounced than in humans, where the OXC/MHD AUC ratio was approximately 0.02 after administration of either OXC or MHD. MHD is primarily eliminated by glucuronidation in humans. These differences in metabolism obviously have important implications for the preclinical toxicological evaluation of OXC.

As a result of the metabolic differences described, animal test species were exposed to much lower levels MHD than would be expected in humans (**Table IIF.1**). (Only Cmax data are available in dogs administered OXC, and the exposures achieved in dogs administered MHD are unknown). At the highest non-lethal dose of MHD administered to rats in repeated-dose studies (600 mg/kg), even the combined (MHD + OXC) exposures were well below those expected in patients (**Figure VIII.3**). Although dose spacing was inappropriate in this study (600 to 2000 mg/kg in 13-week), it does not appear that much higher doses could

have been used in the chronic rat and dog studies, particularly those in which MHD was administered, due to limiting toxicity. So it is not clear what more the sponsor could have done under the circumstances. One possibility that does not seem to have been adequately explored is the use of nonhuman primates for toxicity testing, since early PK studies indicated that baboons metabolized the drug like humans, but this species would not be feasible for lifetime studies. There was of course no safety margin between plasma concentrations associated with toxicity in animals and clinical plasma levels. Since much of the toxicity produced by OXC and MHD in animals involved the liver, it was argued that exposure of the liver is more important than total systemic exposure and that the liver burden would be greater in animals due to differences in drug metabolism. The sponsor suggested that administered dose would provide a better index of the amount of drug transformed by the liver and thus would be a more suitable parameter for making animal to human comparisons than plasma concentrations. But in rats, plasma concentrations of MHD and OXC were higher in females than in males at the same dose, presumably due to a higher rate of clearance in males, and liver (as well as other) effects were generally seen at lower doses and with greater incidence and severity for the same dose in females (although the differences in toxicity were not always as striking as might have been expected given the magnitude of the differences in plasma levels). The mechanistic information needed for selecting the appropriate measure of target tissue dose, which could then be used for extrapolation to humans, is lacking; eg, it is not known whether tissue exposure to parent or metabolite(s), microsomal enzyme induction, or cytotoxicity with concomitant regenerative hyperplasia is critical. And while it might be possible to discount some of the animal toxicity on mechanistic grounds (see below), this reasoning would not apply to all of the effects observed and does not address the problem of too little animal exposure to the relevant chemical species.

Because of their similar structures and pharmacological profiles, it is tempting to assume that the toxicity of OXC and CBZ would also be similar. CNS, hematological, and liver toxicity were consistently observed with both OXC and MHD in rats and dogs (kidney toxicity was also prominent in rats but not dogs). These findings are in fact similar to those seen at comparable doses of CBZ in animals and are also among the most common types of toxicities associated with the clinical use of CBZ (GL Holmes, Carbamazepine Toxicity, In: Levy, Mattson, Meldrum, eds. Antiepileptic Drugs. New York: Raven Press, 1995; 567-579). And like CBZ, both OXC and MHD produced liver tumors and Leydig cell tumors in rodents (relevance to humans uncertain). CBZ is teratogenic in animals and humans, and OXC was teratogenic in rats and embryolethal in rabbits. Cardiac conduction disturbances similar to those seen with OXC and MHD in dogs have also been reported with CBZ in dogs and humans (ibid). Comparable disturbances of fluid and electrolyte balance were produced by acute administration of CBZ, OXC, and MHD in rats, although it is not clear how these rat effects relate to the hyponatremia that has been associated with the clinical use of CBZ and OXC. So while it did not appear from these studies that OXC offers any safety advantage over CBZ, there was also no indication of novel toxicity. A possible exception was the evidence of genotoxicity observed with OXC and MHD in some assays, which has not been reported with CBZ (may not have been as thoroughly evaluated). But overall, the toxicity associated with OXC or MHD in animals was quite similar to that seen with CBZ, although, as previously discussed, the possibility that new effects would emerge at higher levels (or ratios) of exposure to MHD cannot be ruled out.

In view of the shortcomings of the animals models, however, the predictive value of the studies conducted with OXC or MHD may be questioned. The ability of humans to rapidly convert OXC to MHD is thought to explain the decreased ability of OXC to induce drug metabolism compared to CBZ. Conversion of OXC to MHD is thought to be catalyzed by cytosolic reductase enzymes, which are less subject to induction than oxidative enzymes, and *in vitro* data indicate that MHD is a weaker inducer of drug metabolism in human hepatocytes than OXC or CBZ. Because of the metabolic differences described, this potential advantage cannot be readily evaluated in animals. Hepatic enzyme induction and its presumed toxicological sequelae (including secondary effects, such as endocrine-related findings) were seen with both OXC and MHD in rats. Limited TK data did not indicate induction of metabolism after repeated administration of OXC (2-week po study) or MHD (13-week iv study) to dogs, and while some hepatotoxicity was observed in the chronic toxicity studies of OXC and MHD in dogs, the effects on the liver were less pronounced than in rats. The observed species differences in the elimination of OXC could also have toxicological consequences. In humans, OXC and its metabolites are excreted almost exclusively via the kidneys; in rats, biliary excretion predominates; and

in dogs, renal excretion approximately equals fecal recovery after oral administration. So, due to major species differences in disposition, the relevance to humans of much of the toxicity found in animal studies of OXC and MHD (e.g., hepatobiliary, gi effects in rats) is uncertain.

Based on the animal data, there is no evidence that OXC will produce any major toxic effects not associated with its chemical analogue CBZ. This agrees with clinical data showing comparable types and incidences of side effects when the two agents were compared in the same study (Dam et al., Epilepsy Res 3:70-76, 1989). The most commonly reported adverse effects associated with OXC, such as CNS toxicity (dizziness, sedation, fatigue, visual disturbances, ataxia) and changes in hematological parameters (WBCs, platelets, RBCs) and liver function tests (ALT, ALP, GGT), are very similar to those seen with CBZ (Dam and Ostergaard, Other Antiepileptic Drugs - Oxcarbazepine, In: Levy, Mattson, Meldrum, eds. Antiepileptic Drugs. New York: Raven Press, 1995; 987-995). There has been some indication of fewer hypersensitivity reactions (skin rash) with OXC than with CBZ, although there is not enough data to assess the relative frequencies of more serious responses. While both drugs have been shown to induce hyponatremia, there is evidence that this effect may be more pronounced with OXC (ibid). Hyponatremia has been proposed as a possible mechanism for CBZ teratogenicity based on the finding that when rat embryos were cultured with serum from patients treated with CBZ, the embryotoxic effects correlated with the degree of hyponatremia (Lindhout et al., In: Nau and Scott, eds. Pharmacokinetics in teratogenesis. Boca Raton, FL: CRC Press, 1987:233-50). A retrospective study following-up 947 Danish patients treated with OXC identified 12 women who had received the drug during the first trimester of pregnancy (Friis et al., Acta Neurol Scand 87:224-227, 1993). No congenital anomalies were observed, but the rate of spontaneous abortion (3/12; 25%) was higher than that reported in the Danish population (11%) or in women with epilepsy (12.6%). In another series of 11 prospectively monitored pregnancies in women treated with OXC, alone or in combination with other AEDs, infants with spina bifida, cephalothoracic disproportion, hypospadias, and limb defects were identified (Lindhout and Omtzigt, Epilepsia 35 (Suppl 4):S19-S28, 1994). Although the numbers are too small to draw any conclusions about risk, these findings in combination with the animal data indicate that OXC should not be assumed to be a safe alternative to CBZ for use during pregnancy. This should be addressed in labeling.

Thus, because the animal models employed were less than ideal from the standpoint of comparative metabolism, the preclinical studies submitted must be considered of somewhat limited value in making a safety assessment of oxcarbazepine; and there is no obvious practical remedy for the inadequacies in the preclinical evaluation. This deficiency becomes less crucial, however, when the studies are viewed in the context of previous experience with CBZ and OXC. Taken together, the available information indicates that this drug will be reasonably safe if properly labeled.

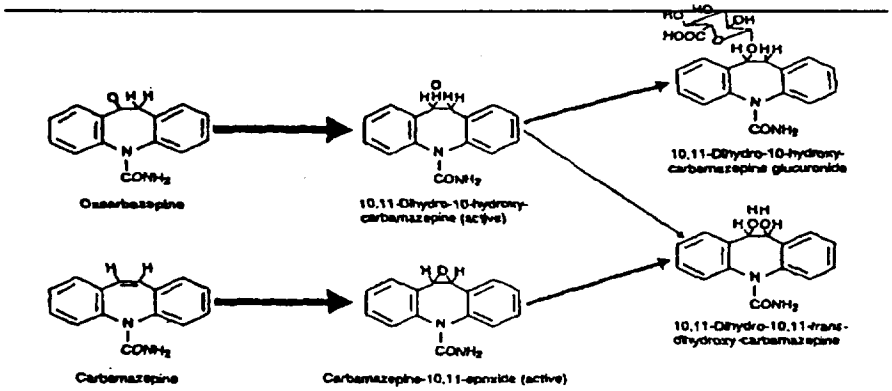


Figure VIII.1 Metabolism of oxcarbazepine and carbamazepine in humans

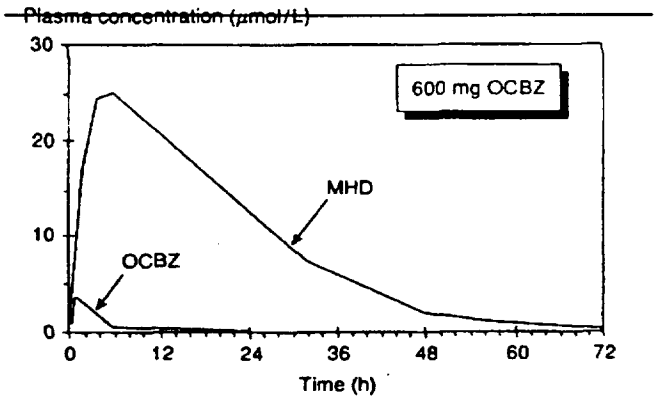


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Figure VIII.2 Plasma concentration-time curves for OXC and MHD in humans after a single dose of OXC

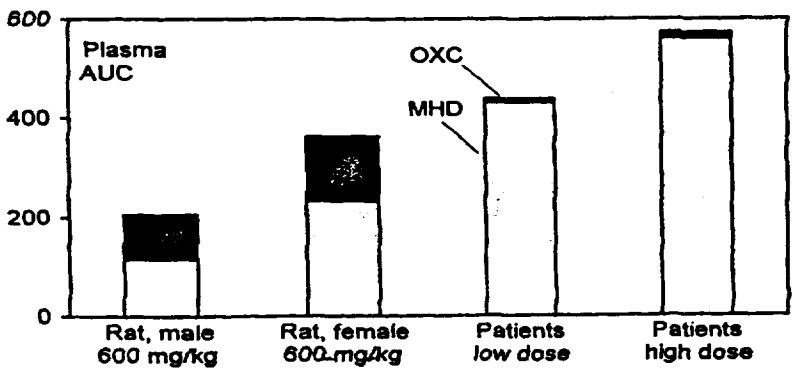

Figure VIII.3 Systemic exposures (AUC) to OXC and MHD in rats dosed with MHD and in patients dosed with OXC

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IX. RECOMMENDATIONS

The NDA is approvable with respect to the pharmacology/toxicology portion. Recommendations concerning the proposed labeling are made in Summary and Evaluation section of the review.

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/S/
J.E. Fisher, Ph.D.

cc:
NDA (21-014)
Div File
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Executive CAC - 7/20/99

Committee: Joseph Sun, Ph.D., HFD-570, Acting Chair
Joseph Contrera, Ph.D., HFD-900, Member
Mark Vogel,, Ph.D., Alternate Member
Glenna Fitzgerald, Ph.D., Team Leader
Ed Fisher, Ph.D., 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.

NDA #: **21-014**
Drug Name: **Oxcarbazepine**
Sponsor: **Novartis**

Background

Oxcarbazepine (Trileptal; GP 47680) is a 10-keto derivative of the antiepileptic drug carbamazepine. In humans, oxcarbazepine is rapidly and almost completely converted to GP 47779, which is present in plasma at levels much greater than (10-60X) those of the parent and is thought to be responsible for most of the drug's antiepileptic activity (anticonvulsant testing indicates that GP 47779 and oxcarbazepine have similar efficacy *in vivo* and *in vitro*). Thus, oxcarbazepine is essentially a pro-drug for GP 47779 in humans. In rodents, however, oxcarbazepine is not converted to GP 47779 to any great extent. In order to more closely approximate human exposure, an additional rat carcinogenicity study was performed with the metabolite. GP 47680 was positive in the Ames test and CHO chromosomal aberration assay. GP 47779 was also clastogenic in the CHO assay.

Mouse Carcinogenicity Study

Dose selection of 10, 40, 70, or 100 mg/kg in the diet for 2 years was based on a 3-month dose range-finding study where significant hepatic toxicity was observed.. There was an apparent treatment-related effect on survival in HD males, but an adequate number survived to termination. BW was not affected in males and was decreased in MD, MHD, and HD females about 10% below C. Liver enzymes (ALT, AST, and ALP) were elevated and histopathology findings in liver, kidney, pancreas and heart were reported in the highest three dose groups. Apparent treatment-related increases in incidences of benign hepatomas were seen in treated males and females. The effect was said to be significant in males (positive trend; HD different from C) but not in females. However, based on the known class effects, the finding of this tumor in both sexes was considered biologically significant.

Rat Carcinogenicity Study

The rat study of GP 47680 was conducted with doses of 0, 25, 75, and 250 mg/kg administered in the diet for 2 years. Dose selection was based on a 6-month toxicity study in which renal and hepatic toxicities were reported. There were no significant treatment-related differences in mortality rates, clinical signs, or clinical pathology parameters; however, BW and food consumption were reduced in HD males (15%) and in MD (15%) and HD (26%) females . A variety of non-neoplastic liver and kidney changes were found histopathologically; these included hepatocellular hypertrophy and vacuolar and cystic

degeneration of hepatocytes in treated males and females, and chronic progressive nephropathy and various associated renal lesions in males and females from all treatment groups. There were apparent dose-dependent increases in incidences of hepatocellular carcinomas in females and testicular interstitial cell tumors in males. Although the increased incidence of hepatocellular carcinoma was not statistically significant, it was considered biologically significant..

In the rat study of GP 47779, doses of 0, 75, 250, and 600 mg/kg were administered by gavage for 2 years. Dose selection was based on a 13-week oral toxicity study where mortality, CNS toxicity , renal, hepatic and hematological toxicities were seen. Clinical signs were noted at the HD and final BWs were significantly reduced in HD males (15%) and MD (12%) and HD (36%) females. In addition, hepatic and renal toxiicities, similar to those seen with GP 47680, were observed at all doses in both sexes. Neoplastic findings consisted of increased incidences of liver tumors in HD males (adenoma) and MD and HD females (adenoma and carcinoma), testicular interstitial cell tumors in MD and HD males, and granular cell tumors of the cervix and/or vagina in females at all doses. Awhile not statistically significant, the increased incidence of tumors in genital organ tumors (interstitial cell and granular cell) were considered biologically significant.

Executive CAC Conclusions and Recommendations:

Mouse study of GP 47680:

(1) The MTD was reached and the study was adequate.
(2) GP 47680 produced hepatoma in both sexes..

Rat study of GP 47680:

(1)The MTD was reached and the study was adequate.
(2) GP 47680 caused hepatocellular carcinomas in females.
(3) GP 47680 produced testicular interstitial cell tumors and should be indicated unless there is an adequate mechanistic explanation, such as hormonal–derived effect .

Rat study of GP 47779:

(1) The MTD was exceeded in females, but because the MD was appropriate the study was valid.
(2) GP 47779 caused hepatocellular adenoma and testicular interstitial cell tumor in males and combined hapatocellular adenona and carcinoma and granular tumor of genital organs in females.

/S/ Sept. 30, 1999

Joseph Sun, Ph.D.
Acting Chair, Executive CAC

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/EFisher, HFD-120
/MMalandrucco, HFD-120
/ASeifried, HFD-024

Note added after meeting of July 20 , 1999:

The committee's comments were given to the statistical reviewer, Roswitha Kelly. When she reanalyzed the tumor incidences in question, there were no changes in her original results. As suspected, the explanation for the apparent discrepancies or failure to reach significance in those cases that the committee commented on had to do with the adjustment for intercurrent mortality. This issue is addressed in detail in an addendum to her review (attached). – Ed Fisher

Addendum to statistical review

In these studies there are several occasions where the gross incidences of tumors (e.g., 0, 3, 2, 6, for control, low, medium, and high dose groups) would suggest a statistically significant finding, but the p value for trend does not reach statistical significance. This phenomenon is basically due to the fact that the test performed by this reviewer adjusts for intercurrent mortality and that the test is the exact permutation trend test, not based on the normal approximation. Adjusting for intercurrent mortality implies not only adjusting for any differential mortality among treatment groups, but also, that the denominators are much less than the total groups size, because the tumors are evaluated within the time intervals in which they occurred. Using an example taken from the second rat study, namely benign granular cell tumors of the vagina, the gross (unadjusted) rates are 0/60, 3/60, 2/60, 6/60. The p-value for the exact permutation trend test (unadjusted for intercurrent mortality) is 0.0128 and for the asymptotic test, it is 0.0089. Both results would be considered statistically significant but the asymptotic results are much more Sc. In reality, most events were found during terminal sacrifice and are based on roughly only half the animals, namely 0/17, 2/24, 2/al, and 6/38 for controls, low, mid and high dose groups. There was one more event in the previous time interval with the following rates: 0/10, 1/13, 0/11, 0/6. The p-value for the exact permutation trend test adjusted for intercurrent mortality is 0.0622, which is not statistically significant. The adjusted asymptotic test has a p-value of 0.0542, which is also not statistically significant, but lower than the one for the exact test. In general, adjusting for intercurrent mortality may decrease the apparent significance of the finding and using asymptotic methods when the incidence of tumors is small tends to give falsely significant results.