

**CENTER FOR DRUG EVALUATION AND RESEARCH**

**Application Number 21-014**

**PHARMACOLOGY REVIEW(S)**

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

**DATE:** September 15, 1999

**FROM:** Glenna G. Fitzgerald, Ph.D.  
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Division of Neuropharmacological Drug Products, HFD-120

**TO:** NDA 21-014  
Trileptal™ (oxcarbazepine)  
Novartis, East Hanover, N.J.  
150,300 and 600 mg. film-coated tablets

**SUBJECT:** Approvability for Pharmacology and Toxicology

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The pharmacology and toxicology studies submitted to this NDA for Trileptal, indicated for use as monotherapy or adjunctive therapy in the treatment of partial seizures in adults and children, are adequate to support its approval. This memo serves to provide my concurrence with the recommendations made by the primary reviewer, Dr. J. Edward Fisher, in his exhaustive review which provides an excellent evaluation of the data included in this rather complex submission. There have been some minor adjustments made to his original labeling recommendations, and final recommended labeling for the pharm/tox sections appears in the action package.

Trileptal is a 10-keto derivative of the antiepileptic drug Tegretol. It was originally postulated that, because Trileptal is reduced to an active monohydroxy metabolite without epoxide formation, it would have a more desirable safety profile than Tegretol. Tegretol follows an oxidative pathway, with formation of an epoxide that has been implicated in some of its more serious toxicities. Actually, the activity and toxicity profiles of Trileptal and Tegretol in animal studies are quite similar.

A major problem that has had to be dealt with during the development of this product has resulted from the fact that the metabolic profile in mice, rats and dogs is not similar to that in humans. In humans the monohydroxy metabolite is the predominant circulating species, while in animals it is the parent drug. In addition to the standard toxicology studies with the parent drug, the sponsor conducted all pivotal studies with the metabolite to address the issue. While not an ideal solution because rats and dogs back-oxidize the metabolite to the parent, those studies did achieve higher levels of metabolite than had been reached in the studies with parent. In general, the toxicities of metabolite and parent appear to be similar. In total, the studies do not provide good

margins of safety, but they do provide us with consistent results and a reasonable evaluation of toxic potential to support an approvable action.

The sponsor has labeled Trileptal Pregnancy Category C, and the data that we have support a C category. Tegretol is category D. There is at least some reason to expect that the risks associated with the two drugs may be similar, so there is concern that the C label could provide some assurance, which is possibly unjustified, that Trileptal is less teratogenic than Tegretol. However, at the present time there is inadequate human data to label this drug other than C. We have therefore inserted a sentence under Pregnancy Category in the label which suggests a relationship to Tegretol.

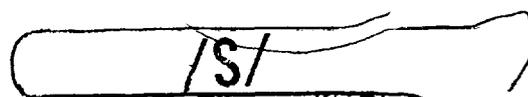
Like Tegretol, hepatocellular tumors occurred in a dose-related fashion in rats dosed with either Trileptal or the monohydroxy metabolite. In the rat study with the monohydroxy metabolite there was also a low incidence of granular cell tumors in the cervix and vagina of rats which the CAC considered to be dose related, and an increase in benign testicular interstitial cell tumors in rats which the CAC considered to be significant.

Because Trileptal will be used in a pediatric population down to the age of 2 years, we requested at the pre-NDA meeting that the sponsor conduct a study in juvenile rats to examine effects on growth and neurological, behavioral and reproductive development. It was agreed that the results could be submitted during Phase 4. That study has recently been submitted, and a cursory review indicates that no unusual effects were observed.

**Recommendations:**

This NDA is approvable for Pharmacology and Toxicology with labeling as it appears in the action package. There are no outstanding issues.

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Glenna G. Fitzgerald, Ph.D.  
Pharmacology Team Leader

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August 20, 1999

Review and Evaluation of Pharmacology and Toxicology  
Original NDA Review

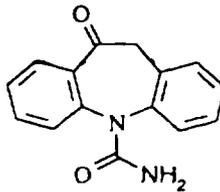
NDA: 21-014

Sponsor: Novartis  
East Hanover, NJ 07936

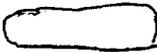
Drug:           Generic Name:       Oxcarbazepine  
                  Trade Name:        Trileptal  
                  Code Name:        GP 47680  
                  Chemical Name:     10,11-dihydro-10-oxo-carbamazepine

Molecular Formula:  $C_{15}H_{12}N_2O_2$   
Mol. Wt.: 252.28

Structure:



Category: Antiepileptic

Related IND: 

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Note: Portions of this review were excerpted from the sponsor's submission.

## I. PHARMACOLOGY (primarily taken from sponsor's summary)

### A. ANTICONVULSANT ACTIVITY IN VIVO

Orally administered GP 47680 (OXC) and GP 47779 (MHD) were compared to standard and new antiepileptic drugs in clinical use in the maximal electroshock seizure (MES) test in rodents (**Table IA.1**). Activity in this model has been shown to predict efficacy against generalized tonic-clonic and partial seizures in man, and it is thought that this model evaluates the capacity of a drug to prevent seizure spread. Other drugs that are primarily active in the MES test, e.g., carbamazepine (CBZ), phenytoin and lamotrigine, also interact with voltage-dependent sodium channels.

**Table IA.1.** Effect of antiepileptic drugs in the MES test in rodents

| <u>Compound</u> | <u>MES test mouse</u><br><u>ED<sub>50</sub> in mg/kg p.o. (range)</u> | <u>MES test rat</u><br><u>ED<sub>50</sub> in mg/kg p.o. (range)</u> |
|-----------------|---|---|
| OXC             | 14.0 (9-20)   | 13.5 (6-13)   |
| MHD             | 20.5(16-37)   | 17.0 (16-22)  |
| Carbamazepine   | 13.0 (11-25)  | 8.0 (5-10)  |
| Phenytoin       | 11.0 (7-20)   | 26.0 (6-36)   |
| Phenobarbital   | 16.5 (13-20)  | 5.5 (4-6)   |
| Valproate       | 220 (160-500)   | 450 (400-600)   |
| Lamotrigine     | 6.1 (4.5-7.7)   | 4.7 (2.3-8.6)   |
| Topiramate      | 35.7(29.2-42.3)   | 17.3(9.9-23.6)  |

The duration of anticonvulsant action (MES test) was about 4 hours for both OXC and MHD as well as for CBZ at the ED<sub>50</sub>. There was no appreciable tolerance to the anticonvulsant effects of OXC and MHD in the MES test in mice and rats over the course of 5 day and 4 week daily administration, respectively.

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 po.

Phenylenetetrazole (PTZ)-induced clonic seizures in mice were blocked by OXC and MHD at doses higher than those needed in the MES test (ED<sub>50</sub>: 30-52 mg/kg po). As seen with carbamazepine and phenytoin, U-shaped dose-response curves were obtained: maximal protection was seen at 60-100 mg/kg, whereas the rate of protection was decreased at a dose of 300 mg/kg. In the sc PTZ test in mice, latencies for convulsions and death were significantly increased with 30 and 100 mg/kg of OXC and MHD. The sc PTZ test generally evaluates the ability of potential antiepileptic drugs to prevent clonic seizures and may also correlate with activity against absence seizures.

Mice were protected from lethal convulsions produced by ip strychnine and clonic convulsions induced by ip picrotoxin only at high doses of OXC and MHD (ED<sub>50</sub>s = 110-300 mg/kg po; 1 hr pretreatment).

OXC and MHD did not significantly influence rat kindling development at doses of up to 100 mg/kg po. This was thought to indicate that they have a lower potential to increase the occurrence of spike-wave/polyspike complexes than phenytoin or carbamazepine. Antiepileptic drugs effective against clonic/absence-type seizures (eg, ethosuximide, valproate) delay rat kindling development (which, in its first stages, includes spike-wave/polyspike complexes in the EEG), whereas phenytoin or carbamazepine are equivocally effective or even enhance kindling evolution. Single-dose studies showed that OXC and MHD (50/100 mg/kg po; 20 mg/kg im) abolished the occurrence or reduced the duration and severity of alumina gel-induced chronic (focal) seizures in Rhesus monkeys. In multiple-dose studies OXC (100 mg/kg po; 30-60 mg/kg im) reduced or suppressed partial seizures in most monkeys. MHD was effective at 100 and 150 mg/kg im.

In rats aged 7, 12, 18, 25, and 90 days, OXC and MHD (5-60 mg/kg ip) did not affect the incidence of clonic seizures induced by PTZ (100 mg/kg sc) but suppressed tonic seizures in all age groups. This parallels the findings in the MES test and indicated that the anticonvulsant properties of OXC and MHD are comparable across age groups.

Comparison of the enantiomers of GP 47779 indicated that S(+) is slightly more potent than R(-) 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 effect profiles).

**Table IA.2.** Anticonvulsant ED50 values of GP 47779 and its enantiomers in the MES test in rodents

| <u>Compound</u> | <u>MES test, mouse</u><br><u>ED<sub>50</sub> in mg/kg p.o.</u> | <u>MES test, rat</u><br><u>ED<sub>50</sub> in mg/kg p.o.</u> |
|-----------------|--|--|
| racemate        | 17/21*   | 21   |
| R (-)           | 35   | 27   |
| S(+)            | 21   | 16   |

\* ED50 values from 2 parallel experiments  
10 animals per dose group. Pretreatment period: 1 h.

In parallel experiments in mice, the therapeutic index (ED<sub>50</sub> for performance impairment in the rotarod test or in the test de la traction by the ED<sub>50</sub> in the MES test) was obtained for OXC, MHD and other antiepileptic drugs (Table IA.3).

**Table IA.3.** Therapeutic index values for OXC, MHD, and other antiepileptic drugs in mice

| <u>Compound</u>  | <u>Rotarod test*</u> | <u>Test de la traction*</u> |
|------------------|----------------------|-----------------------------|
| OXC              | 8.8 (91.3/10.4)      | 12.6 (130.9/10.4)           |
| MHD              | 14.6 (305.9/21.0)    | 16.6 (348.4/21.0)           |
| Carbamazepine    | 7.1 (97.4/13.7)      | 8.9 (121.8/13.7)            |
| Topiramate       | 19.8 (708.5/35.7)    | 16.4 (585.8/35.7)           |
| Lamotrigine      | 6.5 (39.7/6.1)       | 9.9 (60.4/6.1)              |
| Valproate sodium | 1.4 (535.5/384.1)    | 1.3 (517.9/384.1)           |

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\* ED<sub>50</sub> values in the behavioral and MES tests in parentheses.  
10 mice per dose. Pretreatment period: 1 h.

## B. ANTICONVULSANT ACTIVITY IN VITRO

The following *in vitro* studies demonstrated anticonvulsant effects:

- MHD and OXC limited sustained high frequency repetitive firing (SRF) of action potentials in cultured neurons.
- MHD and lamotrigine decreased the field potential amplitude in rat neocortical slices at concentrations of  $3 \times 10^{-6}$  to  $2 \times 10^{-4}$  M in the presence or absence of magnesium. This was taken as evidence that the effect was not mediated by NMDA, unlike the case with felbamate which was effective only in magnesium free solution.
- MHD (from  $3 \times 10^{-6}$  to  $10^{-4}$  M) inhibited glutamatergic EPSPs (intracellular studies using striatal neurons in corticostriatal slices) and OXC inhibited the veratridine-stimulated release of glutamate and other neurotransmitters (rat brain slices; IC<sub>50</sub> =  $4 \times 10^{-5}$  M). However, microdialysis measurements of extracellular glutamate and aspartate in conscious rats showed a weak inhibition

of veratridine-induced glutamate release at the maximally effective anticonvulsant dose of OXC. Similar results were obtained with carbamazepine and lamotrigine. This was thought to indicate that the anticonvulsant effects of these drugs are not mediated by inhibition of glutamate release elicited by ongoing neuronal electrical activity.

- When MHD and its R(-) and S(+) enantiomers were tested for anticonvulsant activity in an *in vitro* system, which minimized the possibility of metabolic reactions including oxidation to OXC, epileptiform discharges induced by penicillin in rat hippocampal slices were suppressed equally well and in a concentration-dependent manner ( $10^{-4}$  to  $5 \times 10^{-4}$ ) by MHD, R(-) and S(+). These findings support the conclusion drawn from *in vivo* tests that the racemate and both enantiomers have a similar anticonvulsant profile. OXC was not tested in this system, however.

### C. MECHANISM OF ACTION

Three possible anticonvulsant mechanisms of action were proposed for OXC and/or MHD (of the three, the first was regarded by the sponsor as being the most important):

- blockade of voltage-dependent sodium channels,
- decrease of high-voltage activated calcium currents, and
- interaction with potassium channels.

At therapeutic concentrations, OXC and MHD limited sustained high frequency repetitive firing (SRF) of sodium-dependent action potentials in cultured mouse neurons. This effect, which is also seen with carbamazepine, phenytoin and lamotrigine, is thought to be involved in the ability to block the spread of seizure activity from an epileptic focus. OXC and MHD displayed similar activity in this model, with EC<sub>50</sub>s of  $5 \times 10^{-6}$  and  $2 \times 10^{-6}$  M, respectively. MHD at concentrations of  $3 \times 10^{-6}$  to  $10^{-4}$  M also decreased high-voltage activated calcium currents in isolated striatal neurons and cortical pyramidal cells. However, MHD did not influence L-type calcium currents at  $3 \times 10^{-4}$  M in patch-clamp studies on rat dorsal root ganglia cells. Despite a weak effect of OXC in this test, the evidence for an interaction with calcium was considered equivocal. In *in vitro* studies in rat hippocampal slices, MHD and its enantiomers suppressed penicillin-induced spiking. This effect was antagonized by  $2 \times 10^{-5}$  and  $2 \times 10^{-4}$  M concentrations of the potassium channel blocker 4-aminopyridine, indicating that the anticonvulsant activity of MHD and its enantiomers may be mediated also by interactions with potassium channels.

At a concentration of  $10^{-5}$  M, OXC and MHD did not bind to brain neurotransmitter or modulator receptor binding sites, with the exception of adenosine receptors for which they showed moderate affinity. Interactions with adenosine receptors may contribute to the psychotropic rather than the anticonvulsant properties of the drugs, because in tests of a series of structurally similar compounds, there was no correlation between anticonvulsant activity and adenosine receptor binding. MHD at  $6 \times 10^{-5}$  M did not affect the presynaptic inhibitory action of adenosine, indicating that presynaptic adenosine receptors were not involved in the MHD-mediated reduction of corticostriatal excitatory postsynaptic potentials.

### D. SAFETY PHARMACOLOGY

No effects on blood pressure, heart rate, blood flow, or ECG were seen in male rabbits administered intravenous doses of 1, 3, and 10 mg/kg OXC or MHD. A po dose of 100 mg/kg OXC produced no effects on blood pressure, heart rate, or ECG in conscious male dogs with normal baseline ECGs. However, in one dog with what were thought to be pre-existing ECG abnormalities (fluctuations in P wave amplitude and ventricular extrasystoles were seen predosing), OXC administration exacerbated the ECG anomalies. In anesthetized cats, OXC produced a small, transient decrease in blood pressure at an iv dose of 10 mg/kg (1/3 iv LD). Intravenous administration of 10, 25, and 50 mg/kg synthesis 2 MHD (sequential infusion; 5 ml/min; 45 min between doses) to anesthetized male dogs produced no effects on blood pressure or heart rate but increased P-wave amplitude and duration at all doses (significant after the HD) compared to C. When the MHD enantiomers were evaluated in anesthetized male dogs (10, 25, and 50 mg/kg; sequential iv infusions), administration of the S(+) enantiomer (CGP 13751) produced a dose-dependent, statistically significant increase in the P-R interval, which lasted for up to 30 min after infusion of the HD. The R(-) enantiomer (CGP

13698) produced the same effect, beginning during administration of the HD and lasting for at least 45 min after the infusion ended. It was suggested the ECG alterations could have resulted from changes in electrolyte levels, as demonstrated in rats (below).

When the renal effects of OXC and MHD were studied in rats, dose-dependent increases in urinary electrolyte excretion were generally seen at oral doses of 30-300 mg/kg. Plasma sodium levels were significantly decreased by the more potent S(+) enantiomer of MHD at oral doses of 100 and 300 mg/kg (122.2 and 122.6 mmol/l, respectively, compared to control range of 140 -142.9 mmol/l). When OXC and MHD were administered to water-loaded rats, significant increases in sodium, potassium, and chloride excretion (up to 6-fold C) and urine osmolality (up to 1.5-fold C) were seen after oral doses of 100 and 300 mg/kg. As was the case with CBZ, little or no effects were seen with OXC and MHD (300 mg/kg) in Brattleboro rats lacking endogenous vasopressin (ADH), leading the sponsor to propose a central mechanism, ie, one involving increased production or release of ADH. This does not seem to necessarily follow, since the drugs could be altering the renal response to ADH; but they do not appear to be acting independently of ADH. However, while electrolyte disturbances were sometimes seen, hyponatremia was not consistently observed in the repeated-dose toxicity studies in rats or dogs, indicating that these species may not be good models for humans.

## II. ADME (primarily taken from sponsor's summary)

### A. ABSORPTION

The kinetics of OXC were examined in the rat and dog following single iv and oral doses of 5 mg/kg of the [<sup>14</sup>C]-labeled drug (studies conducted in 1977). In the rat, it was suggested that the drug was well absorbed since equal amounts of radioactive material were recovered in the urine after iv (46%) and oral (47%) administration. (No distinction is made between drug and metabolites when total urine radioactivity is used, so what is excreted in the urine could represent products of presystemic metabolism. It was found that the amount of unchanged drug in the urine was similar by either route [1.2% of dose], but the low percent of OXC excreted unchanged in rats makes urine analysis inappropriate for assessment of bioavailability). The remainder of the radioactive dose (54-55%) was recovered in the feces (see **Table IIE.1**). In bile fistula rats, 78.7% of an oral dose was excreted in the bile and 12.9% in the urine. The greater urinary excretion of OXC in intact rats was attributed to enterohepatic recirculation. In the dog, incomplete absorption was suggested by the difference in the amounts of total radioactivity excreted in the urine (74% and 49%, respectively) following iv and po administration (unchanged drug in urine <1% of dose by both routes), but when AUCs of unchanged drug were compared, a high bioavailability estimate was obtained ( $AUC_{0-\infty} po/iv = 101\%$ ).

In single oral dose studies in male rats and dogs (5, 30, and 300 mg/kg), AUCs of unchanged drug increased proportionally to dose at up to 30 mg/kg, but were less than expected (~25 and 40%, respectively) at 300 mg/kg (**Table IIB.5**). Non-linearity at this dose level was attributed to decreased absorption.

The kinetics of MHD were also investigated in the rat and dog following single iv and oral doses of 5 mg/kg of the [<sup>14</sup>C]-labeled substance. In the rat, the percentage of the dose excreted in urine was higher after oral (60.2%) than after iv (37.4%) administration, indicating presystemic metabolism following oral administration. However, excretion of unchanged drug in the urine was similar after oral (10.3% of dose) and iv administration (11.5%). In bile fistula rats, 61.5% of an oral dose was eliminated in the bile and 19.1% in the urine (total said to be less than 100% because samples were only collected for 24 hours after dosing). In the dog, complete absorption of the oral dose of MHD was suggested by the agreement between the percentage of dose excreted in the urine and the percentage excreted as intact MHD in the urine after iv (75.1 and 30.8%, respectively) and oral (76.9 and 29%, respectively) administration (**Tables IID.1 and IIE.2**).

Plasma AUCs of unchanged drug measured in male rats and dogs after single oral dose of MHD (5, 30, and 300 mg/kg and 5, 30, and 150 mg/kg, respectively) increased greater than dose-proportionally. The amount of unchanged drug in urine as percent dose showed a similar increase in dogs (**Table IIB.6**). This was thought to indicate either incomplete absorption at the low doses or capacity limited biotransformation and/or distribution.

**B. SINGLE AND MULTIPLE DOSE PHARMACOKINETICS**

**1. Mouse**

In a pilot study, plasma levels of OXC and its metabolites MHD and DHD were determined in male mice after one week of dietary administration of OXC at doses of 30 and 300 mg/kg/day. Mean levels of OXC, MHD and DHD were 0.14, 0.04, and <0.03 ug/ml, respectively, during administration of 30 mg/kg/day (N=5), and 0.89, 0.27, and 0.05 ug/ml, respectively, during administration of 300 mg/kg/day (N= 4).

**2. Rat**

The pharmacokinetics of OXC in plasma were evaluated following iv administration of a single 5 mg/kg dose of [<sup>14</sup>C]-labeled OXC to 4 male rats [Tif:RAIf (SPF)]. Based on AUC(0-24 h) values, intact OXC, MHD and DHD accounted for 45.6%, 3.7% and 0.7%, respectively of the total [<sup>14</sup>C]-labeled substances in plasma (Table IIB.1). Plasma levels of intact OXC declined from 1.99 ug/ml at 5 min to 0.15 ug/ml at 6 h in a non-first-order process; plasma concentrations of MHD ranged from 0.15 ug/ml at 5 min to 0.02 ug/ml at 6 h; and the concentration of DHD was less than or equal to 0.01 ug/ml in all samples.

**Table IIB.1** Proportions of OXC, MHD, and DHD in plasma after iv and/or oral doses of 5 mg/kg of [<sup>14</sup>C]-OXC

| Species | route    | AUC <sub>(0-24 h)</sub> of total radioactivity (ug.h/ml) | % of individual compounds |      |       |
|---------|----------|--|---------------------------|------|-------|
|         |          |  | OXC                       | MHD  | DHD   |
| Rat     | iv (n=4) | 13.6 (100%)  | 45.6                      | 3.7  | 0.7   |
| Dog     | iv (n=2) | 28.8 (100%)  | 14.6                      | 1.7  | 0.9   |
|         | po (n=2) | 16.7 (100%)  | 24.6                      | 3.0  | <0.01 |
| Baboon  | po (n=2) | 9.4 (100%)   | <0.01                     | 35.1 | <0.01 |

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**Table IIB2.** Plasma AUC values for OXC and its metabolites following oral administration of OXC to rats

| Dose (mg/kg) | AUC(0-∞), Day 1 (ug.h/ml) |      |       | AUC(0-24 h), Day 12 (ug.h/ml) |      |       |
|--------------|---------------------------|------|-------|-------------------------------|------|-------|
|              | OXC                       | MHD  | DHD   | OXC                           | MHD  | DHD   |
| 10           | 12.8                      | 1.44 | 0.00* | 14.5                          | 1.44 | 0.00* |
| 50           | 83.5                      | 9.54 | 0.71  | 36.1                          | 2.57 | 0.00* |
| 100          | 143                       | 19.5 | 1.56  | 58.8                          | 4.26 | 0.48  |
| 200          | 343                       | 46.9 | 4.77  | 74.2                          | 3.81 | 0.28  |

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\*: Plasma concentrations were below the limit of quantitation (0.027 ug/ml).

Plasma concentrations of OXC and its metabolites were evaluated after OXC was administered by oral gavage to male rats at doses of 10, 50, 100 and 200 mg/kg. At each dose level, a single dose was given to 4 rats and multiple doses were given once daily for 12 days to another 4 rats. Doses of up to 100 mg/kg were absorbed rapidly, with median T<sub>max</sub> values ranging from 0.75 to 2.5 h. Absorption of the 200 mg/kg dose was slower, with median T<sub>max</sub> values of 7 and 6 h on Days 1 and 12, respectively. After administration of a single dose, the AUC(0-∞) values for OXC were roughly proportional to the dose (Table IIB2). The ratio of the mean

AUC(0-24 h) after repeated dosing to the mean AUC(0-∞) after a single dose decreased from 1.13 at the 10 mg/kg dose to 0.22 at the 200 mg/kg dose, indicating a decrease in the half-life after multiple dosing at the higher doses. Similarly, the AUC(0-24 h) values for the metabolites, MHD and DHD, also fell following multiple dosing with 50 to 200 mg/kg OXC, relative to their respective mean AUC(0-∞) values following a single dose of OXC.

The pharmacokinetics of MHD in plasma were evaluated following iv administration of a single 5 mg/kg dose of [<sup>14</sup>C]-labeled MHD to 4 male rats [Tif:RAIf (SPF)]. Plasma levels of intact MHD declined from 2.6 ug/ml at 5 min to 0.13 ug/ml at 3 h in a first-order process, with an elimination half-life of 41 min. Based on AUC(0-24 h) values, MHD, DHD and OXC accounted for 31.5, 13.9 and 17.6%, respectively, of the total [<sup>14</sup>C]-labeled substances in plasma (Table IIB.3). The remaining radioactivity in plasma was not identified.

Plasma concentrations of MHD and its metabolites were evaluated in male rats given MHD orally at doses of 10, 50, 100 and 200 mg/kg. At each dose level, a single dose was given to 4 rats and multiple doses were given once daily for 12 days to another 4 rats. MHD was absorbed rapidly, with median T<sub>m</sub>ax values ranging from 0.5 to 0.75 h. After administration of a single dose, the AUC(0-∞) values for MHD were roughly proportional to dose (Table IIB.4). The ratio of the mean AUC(0-24 h) after repeated dosing to the mean AUC(0-∞) after a single dose ranged from 79% to 67% at doses of 10 to 200 mg/kg/day, respectively, indicating a moderate induction of metabolism after multiple dosing. Similarly, the AUC(0-24 h) values for the metabolites OXC and DHD also fell following multiple dosing with MHD relative to their respective mean AUC(0-∞) values following a single dose of MHD.

**Table IIB.3** Proportions of MHD, OXC, and DHD in plasma after iv and oral doses of 5 mg/kg of [<sup>14</sup>C]-MHD

| Species | route    | AUC <sub>(0-24 h)</sub> of total radioactivity (ug.h/ml) | % of individual compounds |      |      |
|---------|----------|--|---------------------------|------|------|
|         |          |  | MHD                       | OXC  | DHD  |
| Rat     | iv (n=4) | 10.8 (100%)  | 31.5                      | 17.6 | 13.9 |
| Dog     | iv (n=2) | 20.9 (100%)  | 34.0                      | 3.8  | 8.1  |
|         | po (n=2) | 20.8 (100%)  | 25.0                      | 1.9  | 7.2  |

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**Table IIB.4.** Plasma AUC values for MHD and its metabolites following oral administration of MHD to rats

| Dose (mg/kg) | AUC(0-∞), Day 1 (ug.h/ml) |      |      | AUC(0-24 h), Day 12 (ug.h/ml) |      |      |
|--------------|---------------------------|------|------|-------------------------------|------|------|
|              | MHD                       | OXC  | DHD  | MHD                           | OXC  | DHD  |
| 10           | 6.9                       | 7.0  | 2.5  | 5.4                           | 4.9  | 2.0  |
| 50           | 55.7                      | 53.5 | 15.7 | 38.4                          | 25.7 | 11.1 |
| 100          | 72.2                      | 72.2 | 19.4 | 55.7                          | 39.1 | 21.5 |
| 200          | 230                       | 205  | 59.7 | 155                           | 78.5 | 38.7 |

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The pharmacokinetics of the R(-)- and S(+)-enantiomers of MHD were investigated following the administration of single oral doses of the pure enantiomers of MHD to rats. A single oral dose of 160 mg/kg of each MHD enantiomer was administered to three male and three female rats [Tif: RAIF (SPF)] by gavage. Systemic exposure was higher with R(-)-MHD than with S(+)-MHD. The ratio of AUC(0-24 h) values was about

1.8. AUC values for OXC were close to the AUC values for MHD in male and female rats after administration of S(+)-MHD and in male rats after administration of R(-)-MHD, but much lower in female rats after administration of R(-)-MHD. DHD levels in both male and female animals were much higher after administration of R(-)-MHD than after administration of S(+)-MHD. *In vivo* inversion of R(-)-MHD to S(+)-MHD was detected in male rats; however, the AUC value for S(+)-MHD was only 4% of the AUC value for R(-)-MHD following administration of R(-)-MHD. No inversion of the S(+)- to the R(-)- enantiomer was detected in either sex.

The pharmacokinetics of the R(-)- and S(+)-enantiomers of MHD and its metabolites OXC and DHD were also evaluated after single and multiple oral dosing with the racemic compound in the rat (male S-D rats dosed daily by oral gavage with 100 mg/kg MHD). The AUC(0-8 h) for racemic MHD was nearly identical to the sum of the two enantiomers. This demonstrates that the two assays give comparable results. Systemic exposure was higher for R(-)-MHD than for S(+)-MHD following both single and multiple oral MHD administration. Since racemic MHD is almost completely absorbed in the rat and it is unlikely that there is a difference in the absorption of the enantiomers, this suggests 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. This ratio is much higher than that obtained by separate dosing of the enantiomers (ratio = 1.9) or after intravenous administration of racemic MHD (ratio = 1.1 to 1.8) suggesting that one or both of the enantiomers may alter the other's first pass elimination when they are dosed together. Induction of metabolic enzymes after repeated oral MHD administration to rats is presumably responsible for the observed decrease in MHD and DHD AUC(0-8 h) values from Day 7 to Day 14. Induction of metabolism may also have been responsible for the increase in the AUC(0-8 h) values for OXC after repeated dosing, since the formation of OXC is an oxidative process which may be inducible. The R(-)/S(+) AUC(0-8 h) ratio increased from 6.8 on Day 1 to 12.5 on Day 14, suggesting that S(+)-MHD may be more susceptible to metabolic enzyme induction than R(-)-MHD.

In humans, plasma concentrations of the S(+) enantiomer are greater than those of R(-)-enantiomer by a ratio of about 4:1 following administration of a single oral dose of OXC.

### 3. Dog

Following iv dosing of 2 male beagle dogs with 5 mg/kg of [<sup>14</sup>C]-labeled OXC, the mean plasma level of intact OXC at the first sampling time (0.5 h) was 2.56 ug/ml. The decline in plasma OXC levels was biphasic, with  $\alpha$ - and  $\beta$ -elimination half-lives of 0.8 and 9.0 h, respectively. Mean plasma levels of MHD declined from a value of 0.16 ug/ml at 0.5 h to 0.005 ug/ml at 24 h. Similarly, mean DHD concentrations declined from a value of 0.012 ug/ml at 0.5 h to undetectable levels (<0.005 ug/ml) at 24 h. Based on AUC(0-24 h) values, intact OXC, MHD and DHD accounted for 14.6, 1.7 and 0.9% of the total [<sup>14</sup>C]-labeled substances in plasma (Table IIB.1).

The pharmacokinetics of OXC and its metabolites were also investigated after administration of single oral doses of OXC to male dogs. Three dogs received doses of 5 and 30 mg/kg and 2 dogs received a dose of 300 mg/kg (gelatin capsules). The mean pharmacokinetic parameters are shown in Table IIB.5. Maximum plasma concentrations of OXC were reached in 4 to 8 h. AUC values for the unchanged drug were dose proportional for the 5 and 30 mg/kg doses. AUC values for the 300 mg/kg dose were somewhat lower than expected. AUC values for MHD were less than 5% of AUC values for OXC. DHE levels were undetectable (<0.05 ug/ml) in all samples. Elimination of OXC from plasma was rapid. More than 90% of the urinary elimination occurred within 24 hours. Less than 2% of the dose was excreted in the urine as intact OXC.

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**Table IIB.5.** Pharmacokinetics of OXC and its metabolites following single oral doses of OXC in dogs

| Compound | Dose | AUC (ug.h/ml) | Cmax (ug/ml) | Tmax (h) | Urinary excretion (% of dose) |
|----------|------|---------------|--------------|----------|-------------------------------|
| OXC      | 5    | 1.77          | 0.25         | 4        | 1.05                          |
|          | 30   | 9.61          | 1.13         | 8        | 1.71                          |
|          | 300  | 61.9          | 8.12         | 4        | 0.42                          |
| MHD      | 5    | -             | <0.1         | -        | 0.12                          |
|          | 30   | 0.08          | 0.03         | 3        | 0.13                          |
|          | 300  | 2.75          | 0.37         | 6        | 0.06                          |
| DHD      | 5    | -             | <0.05        | -        | 0.11                          |
|          | 30   | -             | <0.05        | -        | 0.09                          |
|          | 300  | -             | <0.05        | -        | <0.01                         |

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Following iv dosing of 2 male beagle dogs with 5 mg/kg of [<sup>14</sup>C]-labeled MHD, the mean plasma level of intact MHD at the first sampling time (0.5 h) was 3.17 ug/ml. The half-life of MHD was 84 min. Mean plasma levels of DHD reached a maximum value of 0.25 ug/ml at 3 h and declined to 0.08 ug/ml at 12 h. Mean OXC concentrations reached a maximum value of 0.27 ug/ml at 1.0 h and declined to undetectable levels (<0.01 ug/ml) by 12 h. Based on AUC(0-24 h) values, intact MHD, DHD and OXC accounted for 34.0, 8.1 and 3.8% of the total [<sup>14</sup>C]-substances in plasma (Table IIB.3). The remaining radioactive components in plasma were not identified.

The pharmacokinetics of MHD and its metabolites were also investigated in 3 male beagle dogs given single oral doses of 5, 30 and 150 mg/kg MHD in gelatin capsules. Absorption of MHD was rapid, with maximum plasma concentrations reached in 1 to 3 h. The mean pharmacokinetic parameters are shown in Table IIB.6. AUC values for the unchanged drug showed a disproportionately large increase at a dose of 150 mg/kg indicating an increase in the half-life (probable capacity limited metabolism) at this dose. The amount of unchanged drug in urine as percent dose showed a similar increase. Some MHD was metabolized to OXC in dogs, but much less than in rats. Elimination of MHD from plasma was rapid; more than 90% of the urinary elimination occurred within 24 h.

**Table IIB.6.** Pharmacokinetics of MHD and its metabolites following single oral doses of MHD in dogs

| Compound | Dose | AUC (ug.h/ml) | Cmax (ug/ml) | Tmax (h) | Urinary excretion (% of dose) |
|----------|------|---------------|--------------|----------|-------------------------------|
| MHD      | 5    | 3.61          | 1.47         | 1        | 4.49                          |
|          | 30   | 37.0          | 12.2         | 2        | 6.99                          |
|          | 150  | 437           | 83.4         | 2        | 22.3                          |
| OXC      | 5    | 0.84          | 0.21         | 1        | <0.18                         |
|          | 30   | 4.72          | 1.14         | 2        | <0.04                         |
|          | 150  | 59.6          | 10.5         | 4        | <0.01                         |
| DHD      | 5    | 2.08          | 0.27         | 2        | 5.43                          |
|          | 30   | 9.78          | 1.17         | 3        | 4.74                          |
|          | 150  | 68.7          | 8.12         | 6        | 4.34                          |

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The pharmacokinetics of the R(-)- and S(+)-enantiomers of MHD were investigated following administration of single oral doses of MHD to beagle dogs. When single oral doses of 50 mg/kg of each pure enantiomer were administered to three male dogs, systemic exposure was higher for R(-)-MHD than for S(+)-MHD. The mean ratio of AUC(0-24 h) values for R(-)- versus S(+)-MHD was 1.7. Both enantiomers were metabolized into OXC and DHD to a low extent. The mean AUC(0-24 h) values for OXC represented 8 and 12% of the AUC(0-24 h) values for the R(-)- and S(+)-enantiomers, respectively. The mean AUC(0-24 h) values for DHD represented 19 and 3% of the AUC(0-24 h) values for the R(-)- and S(+)-enantiomers, respectively. No enantiomer of the opposite configuration was found in plasma after administration either enantiomer.

#### 4. Baboon

Following oral administration of a 5 mg/kg dose of [<sup>14</sup>C]-labeled OXC to 2 male baboons, the unchanged drug and DHD were undetectable in all plasma samples. In contrast, MHD reached plasma concentrations of 0.27 ug/ml at 3 h and 0.24 ug/ml at 24 h. Based on AUC (0-24h) values, MHD accounted for 35.1% of the total [<sup>14</sup>C]-labeled substances in plasma (Table IIB.1).

### C. TISSUE DISTRIBUTION

#### 1. Mouse

The distribution of radioactivity in mice following iv dosing of a 5-mg/kg dose of [<sup>14</sup>C]-labeled OXC was investigated by quantitative radiometry of the organs and tissues and by whole-body autoradiography. One minute after dosing, total radioactivity in blood was 5.8 ug/ml. Higher amounts (9.2 - 13.4 ug/g) were found in the adrenals, kidneys, Harder's gland, thyroids and liver. Lower levels were found in the testes, eye and white fat (1.5 -2.3 ug/g). After 2 hours, levels of radioactivity ranged from 0.6 ug/g (white fat) to 2.3 ug/g (adrenals), 3.1 ug/g (thyroids), 4.0 ug/g (liver), 8.3 ug/g (kidneys) and 68.3 ug/g (bile). The high levels of radioactivity in kidneys and bile indicate extensive renal and biliary excretion.

The distribution of MHD was studied in male albino mice [Tif: MAGf (SPF)] and male pigmented mice [C<sub>3</sub>H/Bom (SPF)] by whole-body autoradiography following a single iv dose and in pregnant albino mice after an oral dose of 5 mg/kg. Following iv administration of [<sup>14</sup>C]-MHD to male mice, radioactivity was rapidly and evenly distributed in most tissues. Uptake of radioactivity into the brain and spinal cord was low and delayed. Concentrations of radioactivity in the blood and most tissues were already declining between 5 and 30 minutes, while concentrations in the brain and spinal cord were still increasing. Thirty minutes after the injection, radioactivity in all tissues declined rapidly. The distribution pattern in pigmented mice did not differ from that in albino mice.

#### 2. Rat

Distribution of radioactivity was investigated in albino rats by radioanalysis of tissues dissected between 5 minutes and 72 hours after iv administration and 168 hours after oral administration of [<sup>14</sup>C]-labeled OXC. Five minutes after iv administration, the highest tissue concentrations of total radioactivity were found in liver (17.64 ug/ml), adrenals (15.00 ug/ml), kidneys (12.32 ug/ml) and lacrimal gland (10.41 ug/ml). Levels in blood and plasma were 5.07 and 4.73 ug/ml, respectively. The concentrations in nerve and brain were 3.7 and 4.1 ug/ml, respectively. After 5 minutes, radioactivity declined gradually to low levels (< 0.05 ug/ml) at 72 hours in most tissues except the thyroid, optic nerve, liver, skin and kidney (0.09-0.31 ug/ml). Concentrations of total radioactivity measured 168 hours after oral dosing were between 0.01 ug/g (lacrimal gland, testes) and 0.30 ug/g (optic nerve).

The disposition of [<sup>14</sup>C]-labeled MHD was investigated in albino rats after iv administration. MHD distributed rapidly to all organs and tissues. Five minutes after injection, the concentrations of radiolabeled substance were highest in the liver, adrenals, and kidneys (10.5, 9, and 7.1 ug/g) and lowest in nerve, testes and brain (<1.5 ug/g). Plasma concentrations of MHD decreased rapidly from 2.6 ug/ml at 5 min to 0.13 ug/ml at 3 h.

Unchanged MHD and metabolically-formed OXC and DHD comprised 31.5, 17.6 and 13.9% of the total AUC for radioactive substances in plasma, respectively.

3. Dog

Residual organ and tissue levels of total [<sup>14</sup>C]-labeled substances were obtained in one dog 96 hours after iv administration. Elimination of [<sup>14</sup>C]-labeled OXC in the dog was somewhat slower than in the rat and mouse. The highest level was found in the retina/choroid of the eye (2.26 ug/g), reflecting an affinity for melanin. Concentrations in the bone marrow, liver, lacrimal gland and lungs, remainder of the eye, thyroids and kidneys ranged from 0.48-0.19 ug/g, while concentrations were < 0.01 ug/g in the remaining tissues.

Following iv administration of 5 mg/kg [<sup>14</sup>C]-MHD to dogs, plasma concentrations of the parent declined from 2.91-3.42 ug/ml at 0.5 h to 0.05-0.09 ug/ml at 8 h. MHD, OXC, and DHD comprised 34.0%, 3.8% and 8.1% of the total AUC for radioactive materials in plasma, respectively. In one dog, 96 h after administration of MHD, residual <sup>14</sup>C concentrations were <0.2 ug/g in all organs and tissues, except for the retina and choroid (5.1 ug/g), the aorta (0.8 ug/g) and the liver (0.3 ug/g).

4. Protein binding

*In vitro* binding of OXC and MHD to rat, rabbit, dog, and human serum proteins was investigated by equilibrium dialysis at 37°C using [<sup>14</sup>C]-labeled compounds. The results are summarized in Table IIC.1. Serum from rat, rabbit, and dog exhibited the same degree of binding of OXC (range 57.7-65.6%) and MHD (range 27.3-27.8%) in the concentration range of 1-10 ug/ml. Binding in human serum at concentrations of 1-100 ug/ml was higher for both OXC (72.5%) and MHD (39.2%).

Table IIC.1 Binding of [<sup>14</sup>C]-labeled OXC and MHD to animal and human serum proteins in vitro

| Species (concentrations) | Binding in %           |                        |
|--------------------------|------------------------|------------------------|
|                          | [ <sup>14</sup> C]-OXC | [ <sup>14</sup> C]-MHD |
| Rat (1-10 ug/ml)         | 57.7 ± 1.4             | 27.3 ± 1.8             |
| Rabbit (1-10 ug/ml)      | 63.5 ± 1.6             | 27.4 ± 0.9             |
| Dog (1-10 ug/ml)         | 65.6 ± 1.9             | 27.8 ± 1.7             |
| Human (1-100 ug/ml)      | 72.5 ± 0.6             | 39.2 ± 1.5             |

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5. Placental transfer

The distribution of MHD was studied in pregnant mice by whole-body autoradiography. Following oral administration of a single 5 mg/kg oral dose of [<sup>14</sup>C]-labeled MHD to pregnant mice, about the same levels of radioactivity were found in the fetuses as in the maternal blood. This indicated that MHD and/or its metabolites crossed the placental barrier. As in the male albino mouse described above, no specific retention of radioactivity was observed in any maternal or fetal tissues. No experimental data are available on the passage of OXC or MHD into the maternal milk of animals.

D. METABOLISM

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1. Rat

The biotransformation of [<sup>14</sup>C]-radiolabeled OXC was investigated in male, bile-duct cannulated rats after administration of a single oral 5-mg/kg dose. The metabolite pattern in bile was qualitatively similar to that in urine. Biliary excretion accounted for 68-74% of the dose. Total excretion of radioactivity in bile and urine

amounted to 82 to 88% of the dose. The total was said to be less than 100% because samples were collected only up to 24 or 48 hours after dosing. The main metabolite in bile and urine was the enolic 0-glucuronic acid conjugate of OXC which accounted for 46% of the dose. Due to the restricted flexibility of the seven-membered azepine ring of OXC, this metabolite existed in two diastereomeric conformations which interconverted spontaneously in aqueous solution. Both enantiomers of MHD were found in bile and urine. The 0-glucuronide of S(+)-MHD predominated over the glucuronide of R(-)-MHD with a ratio of 7:4. The sum of the 0-glucuronides of S(+)- and R(-)-MHD represented 9% of the dose. A further metabolite, which was mainly excreted in urine, was tentatively identified as the enol-O-sulfate of OXC. Another metabolite in bile appeared to be 10,11 -dioxodibenzazepine. DHD was not detected in bile or urine. Free OXC, free MHD, the proposed enol-O-sulfate of OXC and the proposed 10,11 -dioxo-dibenzazepine accounted for 3-6% of the dose each. The total of the assigned metabolite peaks in bile and urine accounted for 70% of the dose. There were no qualitative differences between the metabolite profiles in urine after iv or po dosing.

The metabolism of MHD was studied in albino rats following iv and oral doses of 5 mg/kg of the [<sup>14</sup>C]-labeled compound. On the basis of AUC comparison after IV dosing, intact MHD and the metabolites DHD and OXC accounted for 31.5, 13.9 and 17.6%, respectively, of the total [<sup>14</sup>C]-radioactivity in plasma. The other (37%) radioactive components in plasma were not identified. Intact MHD was the major component identified in the urine and accounted for 10-11% of the dose following both routes of administration (Table IID.1). About 7% of the dose was excreted in the urine as DHD and less than 0.05% as OXC. Treatment of the urine with β-glucuronidase showed that glucuronidation was not an important metabolic pathway for MHD in the rat. The metabolite profile in urine was similar following both iv and oral dosing.

**Table IID.1** Urinary excretion of MHD, DHD, and OXC after iv and oral doses of 5 mg/kg of [<sup>14</sup>C]-MHD

| Species | route | Excretion in % of total urinary radioactivity (% of dose) |            |             |
|---------|-------|---|------------|-------------|
|         |       | MHD   | DHD        | OXC         |
| Rat     | iv    | 27.0 (10.3)   | 17.7 (6.6) | 0.1 (<0.05) |
|         | po    | 19.3 (11.5)   | 12.6 (7.5) | 0.1 (<0.05) |
| Dog     | iv    | 41.1 (30.8)   | 9.7 (7.2)  | 0.5 (0.4)   |
|         | po    | 38.1 (29.0)   | 8.8 (6.7)  | 0.8 (0.6)   |

## 2. Dog

The metabolite profile in dog urine was similar following iv and oral administration of 5 mg/kg of [<sup>14</sup>C]-labeled OXC. Only a small fraction (<2.5%) of the dose appeared in urine as OXC, MHD, or DHD. Treatment of urine with β-glucuronidase did not affect the levels of free OXC, MHD, and DHD in urine, indicating that glucuronidation is not an important pathway for OXC metabolism in the dog. The remaining radioactivity consisted mainly of unidentified metabolites which were more polar than OXC.

The metabolism of MHD was also studied in beagle dogs following iv and oral doses of 5 mg/kg of the [<sup>14</sup>C]-labeled compound. After oral dosing, intact MHD, and the metabolites, DHD and OXC, accounted for 25.0, 7.2 and 1.9%, respectively, of the total radioactivity in plasma. The remaining radioactive components in plasma were not identified. After iv dosing, MHD, DHD and OXC accounted for 34.0, 8.1 and 3.8%, respectively, of total [<sup>14</sup>C]-radioactivity in plasma. 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 (Table IID.1). About 7% of the dose was excreted as DHD and less than 1% as OXC. Treatment of the urine with β-glucuronidase demonstrated that glucuronidation was not an important metabolic pathway for MHD in the dog. The metabolite profile in urine was similar following both iv and oral dosing.

### 3. Baboon

After oral administration of 5 mg/kg of [<sup>14</sup>C]-labeled OXC to baboons, 97% of the radioactive dose was excreted in the urine. MHD was the main radioactive component (47.4%), while OXC and DHD accounted for only 1.2% and 3.2%, respectively, of the radioactivity in urine. Treatment of urine with β-glucuronidase produced small increases in the levels of free MHD and DHD but no change in OXC. Together, all three compounds accounted for about 52% and 60% of total urinary radioactivity before and after enzyme treatment. The remaining radioactivity consisted mainly of unidentified polar metabolites.

### 4. In vitro metabolism

When the *in vitro* biotransformation of OXC by liver slices from rats, mice and hamsters was investigated, the concentration of OXC decreased monoexponentially with liver slices of all species and strains. The mean OXC concentration remaining after 4 hours of incubation was between 53.9% (hamster) and 61.1% (mouse). The reduction of OXC to MHD was low and varied between species and strains. It was highest (7.4%) in the mouse and lowest in Fischer rats (0.5%). The formation of OXC and MHD conjugates was low, if any. DHD was not found either before or after enzymatic treatment with β-glucuronidase/ arylsulfatase. In all incubations, the sum of OXC and MHD accounted for only 55 to 70% of the radioactivity recovered. The remaining portion of radioactivity was associated with several metabolite peaks which were not further identified.

The biotransformation of MHD was studied in the same fashion. The mean MHD concentration remaining after 5 hours of incubation was between 71% (rat TifRAIf) and 82% (mouse). Oxidation of MHD to OXC occurred to a small degree. The mean concentration of OXC at the end of the 5-hour incubation, expressed as a percentage of the initial MHD concentration, was less than 1% for the mouse and hamster and between 2 and 7.6% for the various rat strains. The concentrations of OXC conjugates were similar to those of unconjugated OXC in the respective strains. Conjugates of MHD were formed only in very low amounts, if at all. The mean concentration of DHD at the end of the 5-hour incubation, expressed as a percentage of the initial MHD concentration, was between 0.4% (mouse) and 2.5% (Syrian hamster).

### 5. Humans

Following administration of single oral doses of 400 mg of [<sup>14</sup>C]-labeled OXC to two healthy volunteers, only 2.0% of the integrated total plasma radioactivity (0-72 h; mean value) was attributable to unchanged OXC, but 68.4% to metabolite MHD; the metabolite DHD accounted for 6.4%. 99% of the dose on average was excreted in urine and feces within 10 days, indicating essentially complete elimination. Renal excretion was predominant, amounting to 95.5% of the dose (mean value). The metabolite MHD accounted for 71% of total urinary radioactivity, 28% being present as the free compound and 43% as the glucuronide conjugate (Table IID.2). Only 0.6% was due to unchanged OXC and about 9% to its direct glucuronide. The metabolite DHD and the sulfate conjugate of OXC accounted for 3.6% and 4% of total 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. Comparable data were obtained in an independent study, following single oral doses of 600 mg and 900 mg of unlabeled OXC administered to healthy volunteers.

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**Table IID.2** Parent drug and metabolites measured in plasma and urine following administration of a single oral 400-mg dose of [<sup>14</sup>C]-labeled OXC or MHD to healthy volunteers (mean of two subjects)

| Compound measured | Compound administered : OXC  |                          |                    | Compound administered: MHD   |                          |                    |
|-------------------|------------------------------|--------------------------|--------------------|------------------------------|--------------------------|--------------------|
|                   | Plasma AUC (0-72h) (ug.h/ml) | % of urine radioactivity |                    | Plasma AUC (0-72h) (ug.h/ml) | % of urine radioactivity |                    |
|                   |                              | urine (untreated)        | urine (hydrolyzed) |                              | urine (untreated)        | urine (hydrolyzed) |
| OXC               | 3.2                          | 0.6                      | 10                 | 1.6                          | 0.3                      | 3.0                |
| MHD               | 116.4                        | 28                       | 71                 | 110.2                        | 36.6                     | 83.2               |
| DHD               | 7.0                          | 3.6                      | 3                  | 7.4                          | 5.9                      | 7.0                |
| Total             | -                            | 32.2                     | 84                 | -                            | 42.8                     | 93.2               |

The proposed scheme of metabolic pathways for the biotransformation of OXC in humans involves reduction to the metabolite MHD, followed by glucuronidation of OXC (10-15% of urinary radioactivity), and the formation of a direct sulfate conjugate (4%). Small fractions of the primary metabolite MHD are hydroxylated to yield DHD and its cis-isomer and a metabolite hydroxylated in the aromatic moiety (about 1% and 7%, respectively).

By comparing the areas under the plasma concentration curves (AUC, 0-72 h) following single oral doses of 400 mg of [<sup>14</sup>C]-labeled racemic MHD in another two healthy volunteers, it was determined that 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 (quantitative data in Table IID.2). The recovery of total radioactivity in urine and feces was almost complete within 7 days. In urine, parent MHD covered a much higher percentage of total radioactivity than the two metabolites, DHD and OXC. MHD and its glucuronide conjugate accounted for similar fractions. In sum, parent MHD and the two metabolites represented about 93% of total radioactivity in urine of the two volunteers. The enantiospecific analysis of the free and conjugated MHD revealed a stereoselective reduction of OXC, favoring the S(+)-enantiomer of MHD.

#### 6. In vitro characterization of human OXO reductase

The characteristics of the OXC reducing enzyme was investigated *in vitro* using human hepatic S9 and microsomes. The reductase appeared to be localized mainly in the liver cytosolic fraction, because synthesis of R(-)- and S(+)-MHD per milligram of protein was 2.1-and 6.7-fold higher in hepatic S9 than in microsomes. This reductase preferred NADPH to NADH as a cofactor and had an optimal pH of 5.5 - 6.0. The reducing activity to R(-)-MHD was inhibited by pyrazole (an alcohol dehydrogenase inhibitor) and quercitrin (a carbonyl reductase inhibitor), but not phenobarbital (an aldehyde reductase inhibitor). Reduction to S(+)-MHD was also inhibited by quercitrin, but not pyrazole or phenobarbital. These results suggested that different enzymes may reduce OXC stereoselectively to R(-)- and S(+)-MHD. The Km and Vmax values for the reduction of OXC to R(-)-MHD were 795.9 uM and 0.26 nmol/min/mg protein. A biphasic Lineweaver-Burke plot was obtained for the reduction to S(+)-MHD with the following kinetic values: Km1, 43.8 uM; Vmax1, 0.14 nmol/min/mg; Km2, 1008.4 uM; Vmax2, 0.31 nmol/min/mg. Thus, differences in the AUC values for R(-)and S(+)-MHD may be due to these enzymes having different affinities for OXC.

#### 7. In vitro inhibition of specific human cytochrome P450 enzymes

In order to predict possible drug-drug interactions, the capacity of MHD and OXC to inhibit major cytochrome P450 isozymes was investigated in human liver microsomes *in vitro*. Activities of CYP1A2, CYP2A6, CYP2C9, CYP2C19, CYP2D6, CYP2E1, CYP3A4/5, and CYP4A9/11 were examined, with known inhibitors used as positive controls. Both MHD and OXC were competitive inhibitors of CYP2C19, having Ki values of 88 and 228 uM, respectively. In addition, CYP3A4/5 was inhibited by MHD (Ki=647 uM) and OXC (Ki=270 uM)

in a competitive and non-competitive fashion, respectively. The relatively high  $K_i$  values for the inhibition of CYP3A4/5 by MHD and OXC suggest that these interactions are unlikely to be of clinical importance. On the other hand, therapeutic levels of MHD might inhibit the metabolism of drugs that are substrates for CYP2C19, since the mean dose-normalized (to 600 mg b.i.d.) plasma trough level of MHD during administration of OXC to patients was  $122.2 \pm 31.7$   $\mu\text{mol/L}$  ( $31.1 \pm 8.1$   $\mu\text{g/ml}$ ). However, no drug interaction studies have been conducted in humans using OXC and selective substrates, inhibitors, or inducers of CYP2C19 to verify this conclusion. There is human *in vivo* evidence for an effect of OXC on CYP3A4: co-administration of OXC in humans decreased the AUC values for felodipine and ethinylestradiol, which are known substrates for CYP3A4.

## 8. Enzyme induction

The induction of drug metabolism by MHD, OXC and carbamazepine was studied *in vitro* in cultured human hepatocytes at concentrations of 50 and 200  $\mu\text{M}$ . The results showed that UDP-glucuronyltransferase activity was increased by 22%, 47% and 39% by MHD, OXC and carbamazepine, respectively. Ethoxyresorufin O-deethylase activity was also increased by 69% after a 72-h exposure to carbamazepine. Pentoxyresorufin O-dealkylase activity was not increased by any of the compounds.

In male rats, repeated oral administration of OXC, MHD or carbamazepine (CBZ) at doses of 10, 50, 100 and 200 mg/kg once daily for 12 days led to a phenobarbital-type induction of hepatic enzymes. Treatment with MHD and OXC at the two highest doses caused slightly increased liver weights, indicating a hepatotrophic effect. Biochemically, all three compounds produced clear and dose-dependent induction of liver microsomal cytochrome P450, cytochrome P450-associated monooxygenases, microsomal epoxide hydrolase, UDP glucuronosyltransferase, and cytosolic glutathione S-transferase. In addition, increased microsomal protein content indicated a proliferation of ER membranes. T-R changes in the microsomal content of individual cytochrome P450 isoenzyme proteins included pronounced increases in the levels of CYP2B1 and CYP2B2 as well as moderately increased CYP2C6 levels. The induction of most of the selected biochemical markers by OXC and MHD was apparent at all dose levels from 10 mg/kg to 200 mg/kg.

In a parallel pharmacokinetic study in rats, OXC, MHD and CBZ were administered orally as single and repeated doses of 10, 50, 100 and 200 mg/kg. AUC values for the administered compounds and their main metabolites decreased in a dose dependent manner after 12 daily doses compared with single dosing, indicating auto-induction. It was appeared that the autoinductive effect was weaker with MHD than with OXC or CBZ, since after repeated dosing with 200 mg/kg, plasma AUC values for CBZ, OXC, and MHD were 40.5%, 21.6% and 67.2%, respectively, of the corresponding values obtained after a single dose. 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 (Tables IIB.2 and IIB.4). 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.

Similar results were obtained in a study of the effects of repeated administration of MHD on hepatic drug-metabolizing enzyme activities, liver weights and concentrations of microsomal protein in rats. After oral administration of 80 mg/kg MHD once daily for 4 days, the activity of NADPH-cytochrome C reductase, the hydroxylation of aniline and the N-demethylation of aminopyrine were induced by 21-32%. The rate of p-nitroanisole O-demethylation and the activity of UDP-glucuronyltransferase were increased by 64-71%. The concentration of cytochrome P450 and microsomal protein and the relative liver weight were not significantly different from controls. When the effects of MHD were compared with those of carbamazepine, OXC and phenobarbital given in equimolar doses, the results showed that MHD had a lower inductive potency than the other three compounds.

Toxicokinetic data from a 13-week toxicity study in rats confirmed the auto-inductive effect of OXC. In both sexes, the AUC values for the parent substance decreased by approximately 50% between Days 8 and 68 of daily oral treatment with OXC doses ranging from 100 to 3000 mg/kg (Table IIIA.2). Autoinduction was not

evident in the 2-year rat study of MHD (Table IVC.8). Plasma level data (Cmax) collected in a 2-week oral toxicity study of OXC in dogs did not indicate induction (Table IIIF.1). There are no TK data for repeated oral administration of MHD to dogs, but at the low doses administered in a 13-week iv study of MHD in dogs, there was no evidence of induction.

The major pathway for biotransformation of OXC in humans involves reduction followed by glucuronidation. The enzymes involved in these processes are not thought to be as readily inducible as the oxidative enzymes which seem to play a greater role in the metabolism of OXC and MHD in rats and dogs. According to the sponsor, no changes in the kinetics of MHD were observed in humans during repeated administration of therapeutic doses of OXC.

## E. EXCRETION

OXC is eliminated from humans and animals primarily by hepatic metabolism. Direct excretion of the unchanged compound is insignificant. Table IIE.1 summarizes the cumulative excretion data obtained from the rat, dog and baboon following administration of [<sup>14</sup>C]-labeled OXC. The data are expressed as a percentage of the [<sup>14</sup>C]-labeled dose. In baboons, as in humans, excretion of metabolites is entirely by the renal route, but in dogs and rats there is substantial fecal elimination. The latter is the result of biliary excretion and not an artifact caused by incomplete oral absorption. This is evident by the elimination of total radioactivity in the feces in rats and dogs following intravenous doses (55 and 28% of the dose, respectively) and by excretion in the bile in bile fistula rats following intravenous (60.5%) and oral (78.7%) administration. These radiotracer studies in animals showed that elimination is almost complete within 7 days of oral administration.

The excretion of MHD was also investigated in rats and dogs following single intravenous and oral doses of 5 mg/kg of the [<sup>14</sup>C]-labeled substance (Table IIE.2). Following administration of [<sup>14</sup>C]MHD, excretion of radioactivity was rapid and virtually complete in both rats and dogs. The bulk of the dose appeared in the urine and feces within 72 h after iv and po administration in both species. 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). Presystemic metabolism following oral administration of MHD in the rat may be responsible 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. Apparently, a portion of the dose that is excreted in the bile in intact rats is reabsorbed and excreted in the urine. This would account for the fact that 60.2% of an oral dose of MHD is excreted in the urine of intact rats. The major route of excretion in dogs was via the urine following both oral and intravenous administration (76.9% and 75.1%, respectively). The balance was excreted in the feces.

**Table IIE.1** Cumulative excretion of [<sup>14</sup>C]-labeled OXC after single iv and oral doses of 5 mg/kg

| Species              | Excretion (% of dose) |           |            |           |      |
|----------------------|-----------------------|-----------|------------|-----------|------|
|                      | Baboon (n=2)          | Rat (n=4) |            | Dog (n=2) |      |
| Route                | po                    | iv        | po         | iv        | po   |
| Intact animals       |                       |           |            |           |      |
| Urine (0-144 h)      | 97.0                  | 45.8      | 47.2       | 73.8      | 48.5 |
| Feces (0-144 h)      | 3.7                   | 54.8      | 54.3       | 27.7      | 44.9 |
| Total (0-144 h)      | 100.7                 | 100.6     | 101.5      | 101.4     | 93.4 |
| Bile fistula animals |                       |           |            |           |      |
| Bile (0-24 h)        |                       | 60.5      | 78.7       |           |      |
| Urine (0-24 h)       |                       | 22.3      | 12.9       |           |      |
| Total (0-24 h)       |                       | 82.8      | 91.6 (n=3) |           |      |

**Table IIE.2** Cumulative excretion of [<sup>14</sup>C]-labeled MHD after single iv and oral doses of 5 mg/kg

| Species              | Excretion (% of dose) |      |           |      |
|----------------------|-----------------------|------|-----------|------|
|                      | Rat (n=4)             |      | Dog (n=2) |      |
|                      | iv                    | po   | iv        | po   |
| Intact animals       |                       |      |           |      |
| Urine (0-120h)       | 37.4                  | 60.2 | 75.1      | 76.9 |
| Feces (0-120 h)      | 61.0                  | 39.1 | 24.2      | 21.7 |
| Total (0-120 h)      | 98.4                  | 99.3 | 99.3      | 98.6 |
| Bile fistula animals |                       |      |           |      |
| Bile (0-24 h)        | 42.2                  | 61.5 |           |      |
| Urine (0-24 h)       | 34.2                  | 19.1 |           |      |
| Total (0-24 h)       | 76.4                  | 80.6 |           |      |

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**F. TOXICOKINETICS**

Plasma level data are included with the individual toxicology studies in which they were collected. These data as well as the human exposure data provided are summarized in **Table IIF.1**. (The anticipated clinical dose is 2400 mg). When OXC was administered to rats and dogs, the relative circulating levels of MHD, compared with OXC, were much lower than in humans because reduction of OXC to MHD, which is the primary metabolic pathway in humans, is apparently only a minor route for the metabolism of OXC in these species. Therefore, the MHD exposures achieved in studies in which OXC was administered were much lower than those expected in humans receiving therapeutic doses. The situation was only somewhat improved when MHD was administered to rats, because unlike the human situation, MHD is extensively back-oxidized to OXC in rats. The OXC/MHD plasma AUC ratios were about 1 following administration of MHD to rats. OXC accounted for only 1-2% of the total plasma radioactivity after administration of single oral doses (400 mg) of labeled OXC or MHD to human volunteers; and in other human studies, the OXC/MHD AUC ratio was approximately 0.03 and 0.02, respectively, after administration of OXC and MHD. Plasma level data (C<sub>max</sub>) were only collected in 1 oral toxicity study of OXC in dogs (2-week), and there are no TK data for repeated-dose oral administration of MHD to dogs. However, single-dose studies indicated that back-oxidation of MHD to OXC also occurred in dogs, albeit to a lesser extent than in the rat. When dogs were administered MHD in single oral doses of 5, 30 and 150 mg/kg, the ratio of plasma AUC values for OXC/MHD was between 0.1 and 0.2 (see **Table IIB.6**).

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**Table IIF.1. Exposure after repeated oral administration of OXC or MHD**

| Compound | Species | Duration          | Dose mg/kg             | Sex | OXC          |               | MHD          |               |
|----------|---------|-------------------|------------------------|-----|--------------|---------------|--------------|---------------|
|          |         |                   |                        |     | Cmax (ug/ml) | AUC (ug.h/ml) | Cmax (ug/ml) | AUC (ug.h/ml) |
| OXC      | Mouse   | 2 years (diet)    | 10                     | M   | 0.07         | ND            | <0.05        | ND            |
|          |         |                   |                        | F   | 0.15         | -             | <0.05        | -             |
|          |         |                   | 40                     | M   | 0.30         | -             | 0.06         | -             |
|          |         |                   |                        | F   | 0.27         | -             | <0.05        | -             |
|          |         |                   | 70                     | M   | 0.37         | -             | 0.06         | -             |
|          |         |                   |                        | F   | 0.57         | -             | <0.05        | -             |
|          |         |                   | 100                    | M   | 0.63         | -             | 0.09         | -             |
|          |         |                   |                        | F   | 0.61         | -             | <0.05        | -             |
| OXC      | Rat     | 3 months (gavage) | 100                    | M   | 3.7          | 20.1          | <1.0         | ND            |
|          |         |                   |                        | F   | 6.2          | 49.5          | 0.2          | 0.6           |
|          |         |                   | 300                    | M   | 4.2          | 40.3          | <1.0         | ND            |
|          |         |                   |                        | F   | 7.3          | 90.4          | <1.0         | ND            |
|          |         |                   | 1000                   | M   | 7.7          | 104.7         | 0.5          | 6.1           |
|          |         |                   |                        | F   | 17.4         | 268.4         | 1.3          | 15.1          |
|          |         |                   | 3000                   | M   | 19.3         | 289.7         | 1.1          | 13.8          |
|          |         |                   |                        | F   | ND           | ND            | ND           | ND            |
| MHD      | Rat     | 3 months (gavage) | 50                     | M   | 1.7          | 17.5          | <1.0         | ND            |
|          |         |                   |                        | F   | 3.6          | 24.7          | 2.5          | 9.4           |
|          |         |                   | 200                    | M   | 2.8          | 45.1          | 2.2          | 31.6          |
|          |         |                   |                        | F   | 7.8          | 57.6          | 9.1          | 70.3          |
|          |         |                   | 600                    | M   | 7.5          | 91.7          | 8.4          | 113.6         |
|          |         |                   |                        | F   | 17.0         | 128.5         | 16.3         | 232.6         |
| MHD      | Rabbit  | 5 days (gavage)   | 200                    | F   | 4.2          | 17.0          | 68.4         | 270           |
| OXC      | Dog     | 2 weeks (capsule) | 100                    | M   | 6.92         | ND            | 0.24         | ND            |
|          |         |                   |                        | F   | 4.86         | -             | 0.11         | -             |
|          |         |                   | 300                    | M   | 8.62         | -             | 0.20         | -             |
|          |         |                   |                        | F   | 6.47         | -             | 0.14         | -             |
|          |         |                   | 600                    | M   | 10.9         | -             | 0.34         | -             |
|          |         |                   |                        | F   | 12.9         | -             | 0.36         | -             |
| OXC      | Human   | 5 days            | 10 (600 mg)            | M   | 0.40         | 2.3           | 8.49         | 86.8          |
|          |         |                   |                        | F   | ND           | ND            | 9.34         | 91.5          |
| OXC      | Human   | ≥2 weeks          | 15 (900 mg)            | M&F | 0.65         | 7.7           | 21.7         | 423           |
|          |         |                   | 20-25 (1200 - 1500 mg) | M&F | 1.27         | 12.2          | 27.3         | 532           |

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### III. TOXICOLOGY

#### A. 13-WEEK ORAL TOXICITY STUDY OF GP 47680 (SYNTHESIS 2) IN RATS (Tox ref. 1-22, Report no. T/P (US) 94024 (MIN 931174), conducted by [REDACTED] in 1994, GLP, Vol. 1.38)

##### 1. Methods

Rats (10/sex/group) were dosed with 0 (0.5% CMC), 100, 300, 1000 or 3000 mg/kg by oral gavage for 13 weeks. An additional 5/sex were included in the C and HD groups for assessment of recovery (4 weeks). Endpoints included clinical observations, food consumption and body weight measurements, ophthalmoscopic examinations (weeks -3, 6, and 13), toxicokinetics (5/sex/group/time at 2, 4, 8, and 24 hr after dosing during weeks 2 and 10), clinical laboratory tests (hematology and serum chemistry on all animals and urinalysis on 5/sex/group predose and during weeks 5, 9, and 13 and after recovery period), and gross (all animals) and microscopic (all tissues from C, HD non-recovery males and females, and MHD non-recovery females; gross lesions and target tissues from all groups) pathology. Due to excess mortality in HD females, all recovery C females and the 1 remaining HD recovery female were sacrificed after 13 weeks.

*Strain:* Sprague-Dawley [Tac:N(SD)fBr]

*Drug Batch #:* 000692

*Dose Justification:* Doses were based on the results of a previous 13-week oral (gavage) toxicity studies of GP 47680 (Synthesis 1) in rats using the same doses, ie, 100, 300, 1000, and 3000 mg/kg. Treatment-related effects included increased liver weights, grossly enlarged livers, hepatocellular hypertrophy, and cytoplasmic eosinophilic droplets in hepatocytes at  $\geq 300$  mg/kg; CNS-related clinical signs and decreased BWs at  $\geq 1000$  mg/kg; and monocellular hepatocyte necrosis at 3000 mg/kg.

##### 2. Results

###### a. Mortality and Clinical Observations

T-R deaths were seen at 3000 mg/kg in males (1/15) and at  $\geq 1000$  mg/kg in females (2/10 MHD, 14/15 HD). 15/17 deaths occurred by day 3 of treatment with the rest by day 14. No immediate cause of death was determined at autopsy in these animals, however. T-R clinical observations during the first 2 weeks included ataxia and ptosis in males at  $\geq 1000$  mg/kg and in females at  $\geq 300$  mg/kg, hypoactivity in HD males and in females at  $\geq 1000$  mg/kg, and lacrimation and tremors in HD females. CNS signs were no longer present by the 3rd week.

###### b. Body Weight, Food and Water Consumption

Decreased food consumption and BW gain were seen throughout the treatment period in both sexes at  $\geq 1000$  mg/kg (BW gain 17 and 55% below C in males and 18 and 65% in females at MHD and HD, respectively [only 1 HD female survivor]) and transiently in females at 300 mg/kg (day 14). Final BW was significantly decreased (21% below C) in HD males (lone surviving HD female 19% below C). Water consumption was increased in HD males and in females from all dose groups.

###### c. Ophthalmological Examinations

There were no ophthalmological findings that appeared to be T-R.

###### d. Hematology, Clinical Chemistry, Urinalysis

MCV and MCHB were increased (slight but SS) at all doses in males and at  $\geq 300$  mg/kg in females, and MCHC and % reticulocytes were increased (slight but SS) at  $\geq 1000$  mg/kg in both sexes. HGB and HCT were increased in males at  $\geq 1000$  mg/kg, and RBCs were

decreased at all doses in females on day 29 (up to 10% compared to C; all SS). RBC morphology was altered (echinocytosis) at all doses in both sexes. Prothrombin time was decreased at all doses in males and females, and platelets were increased at  $\geq 100$  mg/kg in females. In recovery animals, MCHB was increased in HD males.

AST was D-D decreased in males and ALP was decreased in females early in the study (SS at all doses at day 29), but at the end of treatment (day 89), ALT and ALP were increased (~50%) in HD males and gamma-GT was increased at  $\geq 1000$  mg/kg in males (HD mean: 24.5 U/L vs 0 in C; both doses SS) and females (HD mean: 29 U/L vs 0.36 in C; SS at both doses). Cholesterol was increased at all doses in males and females (up to 3-fold C; SS at all doses), and glucose was D-D decreased at all doses in males and females (up to 30%; SS at all doses). Total protein, albumin, and globulin were increased at all doses (SS) in males and females. Sodium was increased at  $\geq 300$  mg/kg and calcium was increased at all doses in males and females. Total bilirubin was increased (SS) at  $\geq 300$  mg/kg in males (up to 40%) and at  $\geq 1000$  mg/kg in females (up to 30%). Creatinine was decreased in HD males and at  $\geq 1000$  mg/kg in females. In recovery groups, bilirubin remained elevated in HD males compared to C (no female recovery group).

Urine volume was increased at  $\geq 300$  mg/kg in males and at all doses in females. Proteinuria was seen at all doses in both sexes. Urinalysis was not performed on recovery groups.

e. Organ Weights and Gross Pathology

Liver weights were increased at all doses in both sexes (rel wts. 2.5-fold C at HD). Kidney weights were increased at all doses in males (rel wt ~2-fold C at HD) and in females at  $\geq 300$  mg/kg (1.5X C at HD). Both effects persisted in the recovery group males. Gross observations included enlarged liver in males at  $\geq 1000$  mg/kg, observations of a "tan liver lesion" in a HD female and "pale, rough kidney" in a HD male. Dark lesions and/or roughened mucosal surface were seen in the nonglandular stomach of HD males and females.

f. Histopathology (Table IIIA.1)

Centrilobular hepatocellular hypertrophy was observed with a D-R incidence and severity at all doses in both sexes and persisted in HD recovery males (HD-R). Findings of minimal to moderate hepatocyte vacuolar degeneration (males), minimal single cell necrosis (females), and minimal to moderate focal hepatocellular necrosis (M & F; correlated with tan lesion in 1 HD F) were seen at  $\geq 1000$  mg/kg. In addition, minimal to severe increases in hepatocytic mitotic figures were found in female decedents (those sacrificed moribund by day 3) at  $\geq 1000$  mg/kg. The latter was thought to reflect an early stage of hypertrophy. Nephropathy was seen in males at  $\geq 100$  mg/kg and in females at  $\geq 300$  mg/kg (lower HD F incidence reflects shorter treatment time). It was said to be characterized by the presence of proteinaceous tubular casts, tubular basophilia and basement membrane thickening, dilated tubules, and occasional interstitial inflammation. Although described as similar to early senile nephropathy, the T-R nephropathy was said to be distinguished by the uniformly dilated tubules of the inner cortex/outer medulla, which was the predominant feature in the least affected groups. This finding remained in HD recovery males. In addition, tubular necrosis was found in 1 HD male that died on day 4. Gastric lesions indicative of mucosal irritation (inflammation, ulcer, increased bacteria, basal cell proliferation, hyperkeratosis) were seen in HD males and at  $\geq 1000$  mg/kg in females, primarily in animals sacrificed moribund. Additional findings, considered agonal, included adrenal cortical hypertrophy and lymphocytolysis in HD females.

g. Toxicokinetics

Parent was the predominant species in plasma; GP 47779 AUCs were about 1/20 or less.

Plasma levels of GP 47680 were always about 2-fold higher in females than in males, and levels were higher in both on day 8 than on day 69, indicating autoinduction (Table IIIA.2).

**Table IIIA.1** Summary of histopathology findings in a 13-week oral toxicity study of GP 47680 in rats

| Finding                           | Sex | Group |      |       |       |       |     |      |
|-----------------------------------|-----|-------|------|-------|-------|-------|-----|------|
|                                   |     | 0     | 100  | 300   | 1000  | 3000  | C-R | HD-R |
| <u>Liver</u><br>Hypertrophy       | M   | 0/10  | 9/10 | 8/10  | 9/10  | 10/10 | 0/5 | 2/5  |
|                                   | F   | 0/15  | 9/10 | 10/10 | 10/10 | 15/15 | -   | -    |
| Necrosis,<br>single cell          | M   | 2/10  | 0/10 | 0/10  | 0/10  | 1/10  | 0/5 | 0/5  |
|                                   | F   | 1/15  | 0/10 | 0/10  | 2/10  | 5/15  | -   | -    |
| Necrosis,<br>focal hepatocellular | M   | 0/10  | 0/10 | 0/10  | 1/10  | 1/10  | 0/5 | 0/5  |
|                                   | F   | 1/15  | 1/10 | 0/10  | 0/10  | 3/15  | -   | -    |
| Degeneration,<br>vacuolar         | M   | 0/10  | 0/10 | 0/10  | 1/10  | 1/10  | 0/5 | 0/5  |
|                                   | F   | 0/15  | 0/10 | 0/10  | 0/10  | 0/10  | -   | -    |
| Mitotic increase                  | M   | 0/10  | 0/10 | 0/10  | 0/10  | 0/10  | 0/5 | 0/5  |
|                                   | F   | 0/15  | 0/10 | 0/10  | 2/10  | 7/15  | -   | -    |
| <u>Kidney</u><br>Nephropathy      | M   | 0/10  | 9/10 | 10/10 | 9/10  | 8/10  | 0/5 | 5/5  |
|                                   | F   | 0/15  | 0/10 | 8/10  | 7/10  | 4/15  | -   | -    |
| Necrosis,<br>tubular              | M   | 0/10  | 0/10 | 0/10  | 0/10  | 1/10  | 0/5 | 0/5  |
|                                   | F   | 0/15  | 0/10 | 0/10  | 0/10  | 0/10  | -   | -    |

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**Table IIIA.2** Plasma levels of GP 47680 and GP 47779 after oral administration of GP 47680 to rats

| GP 47680<br>Dose (mg/kg) | Sex | AUC (0-24)<br>(ug*hr/ml) |        |          |        | Cmax<br>(ug/ml) |        |          |        |
|--------------------------|-----|--------------------------|--------|----------|--------|-----------------|--------|----------|--------|
|                          |     | GP 47680                 |        | GP 47779 |        | GP 47680        |        | GP 47779 |        |
|                          |     | Day 8                    | Day 68 | Day 8    | Day 68 | Day 8           | Day 68 | Day 8    | Day 68 |
| 100                      | M   | 38.4                     | 20.1   | 1.1      | -      | 3.9             | 3.7    | 0.1      | -      |
|                          | F   | 113.9                    | 49.5   | 3.4      | 0.6    | 8.0             | 6.2    | 0.4      | 0.2    |
| 300                      | M   | 102.3                    | 40.3   | -        | -      | 8.1             | 4.2    | -        | -      |
|                          | F   | 203.5                    | 90.4   | 0.9      | -      | 16.2            | 7.3    | 0.3      | -      |
| 1000                     | M   | 240.7                    | 104.7  | 10.9     | 6.1    | 16.5            | 7.7    | 0.8      | 0.5    |
|                          | F   | 474.4                    | 268.4  | 27.3     | 15.1   | 35.0            | 17.4   | 1.4      | 1.3    |
| 3000                     | M   | 473.7                    | 289.7  | 23.4     | 13.8   | 26.8            | 19.3   | 1.3      | 1.1    |
|                          | F   | 1121.3                   | -      | 73.9     | -      | 67.6            | -      | 4.6      | -      |

B. 6-MONTH ORAL TOXICITY STUDY OF GP 47680 (SYNTHESIS 2) IN RATS (Tox ref. 1-24, Report no. T/P (US) 97002 (MIN 961019), conducted by Novartis in 1997, GLP, Vol. 1.43)

1. Methods

Rats (15/sex/group) were dosed with 0 (0.5% CMC), 10, 45, or 150 mg/kg by oral gavage for 26 weeks. An additional 10/sex were included in the C and HD groups for assessment of recovery (4 weeks). Endpoints included clinical observations, food consumption and body weight measurements, ophthalmological examinations (predose and wks 13 and 26), toxicokinetics (5/sex/group 2 hr after dosing during weeks 2 and 20), clinical laboratory tests (hematology, serum chemistry, urinalysis on 10/sex/group during weeks 13 and 26 and after recovery period), and gross (all animals) and microscopic (all tissues from C and HD non-recovery; target tissues from all groups) pathology.

*Strain:* Sprague-Dawley (CrI:CD[SD]BR)

*Drug Lot #:* 005693

*Dose Justification:* Doses were based on the results of two 13-week oral (gavage) toxicity studies of GP 47680 (Synthesis 2) in rats. In rats treated for 13 weeks with doses of 100, 300, 1000, or 3000 mg/kg (MIN 931174 above), treatment-related effects included mortality at 3000 mg/kg in males (1/15) and at  $\geq 1000$  mg/kg in females (14/15 HD), clinical signs and decreased BW gain at  $\geq 1000$  mg/kg in males and  $\geq 300$  mg/kg in females, clinical pathology changes indicative of increased RBC turnover ( $\uparrow$  MCV, MCH, reticulocytes, echinocytosis) and altered hepatobiliary ( $\uparrow$  GGT, cholesterol, bilirubin, bile acids) and renal tubular function (polyuria, proteinuria) at all doses, increased liver weights and hepatocellular hypertrophy at all doses, hepatocyte vacuolar degeneration and necrosis at  $\geq 1000$  mg/kg, increased hepatocytic mitotic figures in female decedents at  $\geq 1000$  mg/kg, and nephropathy in males at  $\geq 100$  mg/kg and females at  $\geq 300$  mg/kg (Table IIIA.1, above). Plasma levels of GP 47680 were always about 2-fold higher in females than in males (Table IIIA.2, above). In the second 13-week study (20, 60, and 300 mg/kg), T-R findings included decreased prothrombin time and echinocytosis at 300 mg/kg; increased serum albumin, calcium, and total protein and decreased glucose at  $\geq 20$  mg/kg; increased globulin, total cholesterol and phosphorus at  $\geq 60$  mg/kg; increased total bilirubin at 300 mg/kg; increased liver wts and hepatocellular hypertrophy at  $\geq 60$  mg/kg; and renal tubular damage ( $\uparrow$  kidney wts, proteinuria, nephropathy, polydipsia and polyuria) at 300 mg/kg.

2. Results

a. Mortality and Clinical Observations

There were no T-R deaths. The only T-R observation was an increased incidence of perineal staining in MD and HD females.

b. Body Weight, Food and Water Consumption

There were no treatment effects on BW parameters. A slight, transient decrease in food consumption was seen in HD females. Increased water consumption was observed in MD and HD males.

c. Ophthalmological Examinations (predose and wks 13 and 26)

There were no ophthalmological findings that appeared to be T-R.

d. Hematology, Clinical Chemistry, Urinalysis

RBCs, hemoglobin, and hematocrit were decreased slightly (5-10%; SS) in MD and HD males at 3 months but not thereafter.

Cholesterol was increased in HD males (20%; NS) and MD and HD females (up to 40%; SS).

Globulin was increased slightly in MD and HD females (10-30%; SS), and triglycerides were decreased (50-80%) in HD females. Liver enzymes (AST, ALT, ALP) were consistently decreased in treated females, reaching statistical significance at the MD and HD (AST and ALT decreased by 50% compared to C at 6 months). AST was also significantly lower in HD males at 6 months. Liver enzymes remained lower and globulin remained slightly elevated after the recovery period in HD females (both SS).

Urine volume tended to be increased (up to 60%) in treated males and females, but the effect was neither D-R nor SS.

e. Organ Weights and Gross Pathology

Absolute and/or relative kidney weights were increased in HD males and females (~10% compared to C). Absolute and relative liver weights were increased in HD males (20%) and MD and HD females (30-40% at HD). There were no T-R gross findings.

f. Histopathology

At the end of the treatment period, hepatocellular hypertrophy was found in HD males and MD and HD females. Brown (lipofuscin) pigment deposition was also found in HD females. The incidence of thyroid hypertrophy/hyperplasia was increased in HD males, and incidences of chronic progressive nephropathy were increased very slightly in HD males and females. After the recovery period, hepatocellular hypertrophy was still present in some HD group females (HD-R F).

**Table IIIB.1.** Histopathological Findings in Rats during 6-Month Oral Toxicity Study of GP 47680

| Group                              | C                          |   | LD |    | MD |    | HD |    | C-R |    | HD-R |    |    |   |
|------------------------------------|----------------------------|---|----|----|----|----|----|----|-----|----|------|----|----|---|
|                                    | M                          | F | M  | F  | M  | F  | M  | F  | M   | F  | M    | F  |    |   |
| Kidney<br>chronic nephropathy      | N:                         |   | 15 | 15 | 0  | 0  | 0  | 0  | 15  | 15 | 0    | 0  | 2  | 0 |
|                                    |                            |   | 5  | 2  | 0  | 0  | 0  | 0  | 7   | 3  | 0    | 0  | 2  | 0 |
| Liver                              | N:                         |   | 15 | 15 | 15 | 15 | 15 | 15 | 15  | 16 | 10   | 10 | 10 | 9 |
|                                    | hepatocellular hypertrophy |   | 0  | 0  | 0  | 0  | 0  | 11 | 14  | 14 | 0    | 0  | 0  | 2 |
|                                    | brown pigment deposition   |   | 0  | 0  | 0  | 0  | 0  | 0  | 0   | 4  | 0    | 0  | 0  | 0 |
| Thyroid<br>hypertrophy/hyperplasia | N:                         |   | 15 | 15 | 15 | 0  | 15 | 0  | 15  | 16 | 10   | 0  | 10 | 0 |
|                                    |                            |   | 1  | 1  | 1  | 0  | 2  | 0  | 7   | 1  | 1    | 0  | 1  | 0 |

g. Toxicokinetics

Toxicokinetic analysis showed that levels were higher in females than in males and were higher during week 2 than during week 20 (suggesting autoinduction). Plasma levels of GP 47680 at 2 hr after administration (approximate Tmax) are shown in **Table IIIB.2**. Concentrations of the metabolites GP 47779 and CGP 10000 were negligible in both sexes.

**Table IIIB.2** Plasma levels (mean  $\pm$  SD) 2 hours after oral administration of GP 47680 to rats in a 6-month study

| Dose (mg/kg) | Sex | GP 47680 (ng/ml) |                 |
|--------------|-----|------------------|-----------------|
|              |     | Week 2           | Week 20         |
| 10           | M   | 452 $\pm$ 450    | BQL             |
|              | F   | 1941 $\pm$ 602   | 1119 $\pm$ 322  |
| 45           | M   | 1245 $\pm$ 761   | 825 $\pm$ 630   |
|              | F   | 4591 $\pm$ 2227  | 2793 $\pm$ 980  |
| 150          | M   | 2100 $\pm$ 710   | 1734 $\pm$ 549  |
|              | F   | 7263 $\pm$ 3473  | 4942 $\pm$ 2307 |

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C. 13-WEEK ORAL TOXICITY STUDY OF GP 47779 (SYNTHESIS 2) IN RATS (Tox ref. 2-8, Report no. T/P (US) 95009 (MIN 941092), conducted by [redacted] in 1995, GLP, Vol. 1.54)

1. Methods

Rats (10/sex/group) were dosed with 0 (vehicle: 0.5% CMC), 50, 200, 600, or 2000 mg/kg by oral gavage for 13 weeks. An additional 5/sex/group were included for plasma drug level determinations (at 2, 4, 8, and 24 hr after dosing during week 10). Endpoints included clinical observations, food consumption and body weight measurements, clinical pathology (hematology and serum chemistry on all animals at 13 weeks; urinalysis on 5/sex/group during week 12), and gross (all animals) and microscopic (all tissues from C and HD; gross lesions, liver, and kidneys from all groups) pathology. *Strain:* Sprague-Dawley [CrI:VAF/Plus CD(SD)BR] *Drug Lots #s:* 800294 and 800594

*Dose Justification:* Doses were based on the results of a previous 3-month oral (gavage) toxicity studies of GP 47779 (Synthesis 1) in rats with doses of 200, 600, and 2000 mg/kg. Treatment-related effects in that study included mortality at the HD (1/30), sedation at  $\geq$ 600 mg/kg, decreased BW gain in males at 600 mg/kg and in both sexes at 2000 mg/kg, increased ALT and thrombocytopenia at the HD, increased liver weights and hepatocellular hypertrophy (with intracytoplasmic inclusion bodies) at all doses (marked at HD), hepatocyte necrosis at  $\geq$ 600 mg/kg, and brown pigment in hepatocytes at the HD. Effects on the kidney were not reported.

2. Results

a. Mortality and Clinical Observations

13 HD animals died by day 4 of treatment. 3 male and 7 female deaths were attributed to dosing accidents based on pathology; the remaining 2 male and 1 female deaths were considered T-R. The unusually high death rate was said to be due to "the gritty texture of the HD test suspension." Due to the high rate of mortality in the HD group, surviving HD toxicokinetic animals (1/5 M, 2/5 F) were reassigned to the main study group. Ataxia and hypoactivity were observed in both sexes at doses  $\geq$ 600 mg/kg, and recumbency and inactivity were seen at the HD (incidences slightly higher in females initially, decreased over course of study in both sexes, but more rapidly in F). In addition, salivation and perineal staining were seen at  $\geq$ 600 mg/kg in males and at  $\geq$ 200 mg/kg in females.

b. Body Weight, Food and Water Consumption

Statistically significant reductions in mean body weight gain were seen in males at 600 mg/kg (-9% compared to C) and in both sexes at 2000 mg/kg (-50% M; -20% F). Food consumption was consistently decreased (SS) in HD males and females. Slight (but SS) decreases were seen at some time points in males and females receiving 600 mg/kg. T-R increases in water consumption were seen at all doses.

**Table III.C.1.** Mean body weight changes in 13-week toxicity study of GP 47779 in rats

| GP 47779<br>Dose (mg/kg) | Males            |         |                                | Females          |         |                                |
|--------------------------|------------------|---------|--------------------------------|------------------|---------|--------------------------------|
|                          | Body Weight (gm) |         | Percent<br>Change <sup>1</sup> | Body Weight (gm) |         | Percent<br>Change <sup>1</sup> |
|                          | Baseline         | Week 13 |                                | Baseline         | Week 13 |                                |
| 0                        | 165.6            | 557.1   | 236.8                          | 139.6            | 331.7   | 138.0                          |
| 50                       | 171.7            | 553.5   | 222.4<br>(-2.5)                | 144.9            | 348.3   | 140.7<br>(5.9)                 |
| 200                      | 163.4            | 533.1   | 226.5<br>(-5.6)                | 138.1            | 315.8   | 129.1<br>(-7.5)                |
| 600                      | 165.3            | 523.3   | 217.0*<br>(-8.6)               | 142.1            | 325.7   | 130.3<br>(-4.4)                |
| 2000                     | 167.3            | 371.2   | 116.3**<br>(-47.9)             | 138.8            | 286.3   | 101.5**<br>(-23.2)             |

<sup>1</sup> value in parentheses is percent weight gain relative to control

c. Hematology, Clinical Chemistry, Urinalysis

HGB, MCV, and/or MCHB were increased (5-10%; SS) at ≥600 mg/kg in males, and MCV was increased in HD females. Reticulocytes were increased in HD males and females. D-D echinocytosis (slight to moderate) was seen at all doses in both sexes. WBCs and lymphocytes were decreased in HD males and females, and eosinophils were decreased at all doses in males and in HD females. PT was decreased at ≥600 mg/kg in males

GGT was markedly increased at ≥600 mg/kg in males (HD mean: 19 U/L vs 0.5 in C; both doses SS) and in HD females (HD mean: 17 U/L vs 1.20 in C). Cholesterol was increased at ≥200 mg/kg in males (up to 2.5-fold C) and females (up to 3-fold C). Triglycerides and glucose were decreased (up to 30 and 70%, respectively) at ≥600 mg/kg in both sexes. Total protein and/or albumin and globulin were increased at all doses in both sexes. AST was D-D decreased in males and females, and ALT was significantly decreased in HD females. Sodium was increased somewhat at all doses in males and females, reaching SS in HD females, and calcium and phosphate were increased at ≥200 mg/kg in males and females. Chloride was decreased in HD males and at ≥200 mg/kg in females. Total bilirubin was increased in HD males (40%) and at ≥200 mg/kg in females (up to 40%). Bile acids were increased at ≥200 mg/kg in males and at all doses in females (both sexes up to 6-fold). BUN was decreased in HD males and females (10-20% compared to C in both sexes).

Urine volume was increased at all doses in males and females. Proteinuria was seen at all doses in both sexes.

d. Organ Weights and Gross Pathology

Liver weights were increased at all doses in both sexes (2-2.5-fold C at HD). Kidney weights were increased in HD males (rel wt. 25% above C) and in females at  $\geq 200$  mg/kg (32% at HD). There were no T-R gross observations.

e. Histopathology (Table IIIC.2)

Hepatocellular hypertrophy was observed with a D-R incidence and severity at  $\geq 200$  mg/kg in both sexes (lower HD incidences reflect early deaths). Hepatocellular vacuolization was seen in HD males and females, but there was no increase in hepatocellular necrosis or other liver pathology. Nephropathy, said to be characterized by hyaline casts and tubular dilatation, was increased at  $\geq 600$  mg/kg in males. The failure to see an increase in females was probably due to the early deaths. Other kidney findings included possible increases in pelvic dilatation at the HD and tubular basophilia at  $\geq 600$  mg/kg in males. The gastric lesions seen with GP 47680, were not found in this study. There was evidence of dosing accident in 10/13 HD animals that died or were sacrificed by day 4. An increased incidence of immature reproductive organs in HD males was attributed to the early deaths in this group.

f. Toxicokinetics

Plasma levels were measured at 2, 4, 8, and 24 hr after dosing during week 10. Due to the excess mortality at the HD, surviving HD toxicokinetic animals were reassigned to the main study group, so there were no TK samples at this dose. Plasma levels were always higher in females than in males. Within sexes, Cmax and AUC values were generally similar for GP 47779 and GP 47680, with the exception of AUCs in females at 600 mg/kg (Table IIIC.3).

**Table IIIC.2** Pathology Findings in a 13-Week Toxicity Study of GP 47779 in Rats

| Dose (mg/kg)               | 0  |    | 50 |    | 200 |    | 600 |    | 2000 |    |
|----------------------------|----|----|----|----|-----|----|-----|----|------|----|
| Sex                        | M  | F  | M  | F  | M   | F  | M   | F  | M    | F  |
| N                          | 10 | 10 | 10 | 10 | 10  | 10 | 10  | 10 | 11   | 12 |
| Kidney                     |    |    |    |    |     |    |     |    |      |    |
| nephropathy                | 1  | 2  | 1  | 3  | 1   | 2  | 3   | 1  | 5    | 2  |
| tubular basophilia         | 4  | 0  | 6  | 0  | 4   | 2  | 8   | 0  | 5    | 2  |
| pelvic dilatation          | 2  | 2  | 1  | 3  | 0   | 1  | 1   | 0  | 4    | 3  |
| Liver                      |    |    |    |    |     |    |     |    |      |    |
| hepatocellular hypertrophy | 0  | 0  | 0  | 0  | 6   | 9  | 10  | 9  | 7    | 4  |
| hepatocellular vacuolation | 0  | 0  | 1  | 0  | 0   | 0  | 0   | 0  | 2    | 2  |

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c. Clinical laboratory

Thrombin time was increased (up to 30%) in males and females from all treatment groups. Reticulocytes were increased in MD and HD males (up to 60%) and in HD females (10%) at various times during the study.

Sodium was generally decreased in treated males and females, reaching significance at all doses at various intervals over the course of the study. ALT and ALP were increased in treated females at all doses (up to 1.5 and 2-fold, respectively). ALP remained elevated in treated recovery females (SS at LD and HD compared to C). There were no other differences between recovery groups.

There were no T-R changes in urinalysis parameters.

d. Organ weights and Pathology

Small D-D increases in relative liver, kidney, adrenal, and gonad weights were seen in males (up to 30, 10, 50, and 40% compared to C) and females (up to 40, 20, 30, and 30% compared to C). No T-R gross or microscopic pathological changes were found.

e. Plasma drug levels

Mean levels of GP 47779 and GP 47680 at 1 and 6 months are shown in **Table IIID.1**.

**Table IIID.1** Mean plasma levels of GP 47779 and GP 47680 measured at one time point (7 AM) during dietary administration of GP 47779 to rats (5/sex/group) in a 6-month toxicity study

| GP 47779<br>Dose (ppm) | Sex | Plasma Levels<br>(ug/ml) |         |          |         |
|------------------------|-----|--------------------------|---------|----------|---------|
|                        |     | GP 47779                 |         | GP 47680 |         |
|                        |     | Month 1                  | Month 6 | Month 1  | Month 6 |
| 1000                   | M   | 1.66                     | 0.94    | 1.11     | 0.51    |
|                        | F   | 2.99                     | 2.18    | 2.24     | 2.01    |
| 3000                   | M   | 2.48                     | 1.96    | 1.34     | 1.26    |
|                        | F   | 6.43                     | 4.34    | 4.29     | 3.77    |
| 10000                  | M   | 7.41                     | 3.24    | 3.20     | 1.93    |
|                        | F   | 10.32                    | 8.33    | 5.02     | 4.92    |

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E. PILOT 4-WEEK ORAL TOXICITY STUDY OF GP 47680 (SYNTHESIS 2) IN DOGS (Tox ref. 1-25, Report no. T/P (US) 95079 (MIN 951213), conducted by [redacted] in 1995, GLP, Vol. 1.44)

1. Methods

Beagle dogs (2/sex/group) were dosed with 0 (empty gelatine capsules), 200, 400, or 600 mg/kg orally (capsules) for 4 weeks. Because of limited evidence of toxicity at the HD, the MD was increased to 800 mg/kg on day 22. Endpoints included clinical observations and BW measurements, clinical laboratory tests (hematology and serum chemistry on all dogs predosing, and on days 11 and 25), organ weights (brain, liver, and kidney), and gross (all animals) and microscopic (limited number of tissues from all animals) pathology.

*Drug Batch #:* 000692

*Dose Justification:* Doses were based on results of acute, 10-day, 3-month, and 12-month oral toxicity studies with GP 47680 produced by the old synthetic method. In the acute study, emesis was seen at  $\geq 600$  mg/kg and tachycardia and hyperventilation were observed at 1200 mg/kg. In the 10-day study, CNS signs, decreased BW gain, decreased RBCs, increased liver enzymes (ALT, AST, and ALP), and increased liver weights were seen at 600 mg/kg (only dose tested). In the 3-month study (60, 200, 600 mg/kg), emesis occurred at the HD, there were no effects on BW gain, liver wts were increased at all doses, ALT and AST were increased in 1 HD male, hemosiderin deposits were seen in liver Kupfer cells at the MD or greater, and hemosiderin was found in the kidney at the HD. In the 1 year study (60, 200, 600-400 mg/kg), decreased weight gain and thymic atrophy at the HD were the only effects reported.

2. Results

a. Mortality and Clinical signs

There were no deaths during the study. Clinical observations included CNS signs (ataxia, loss of righting reflex, paresis, recumbency) in 1 female receiving 400 or 800 mg/kg, a slightly increased frequency of vomiting and "vomit with feed and/or apparent compound" at  $\geq 400$  mg/kg, and an increase in the incidence of "feces with apparent compound" at all doses.

b. Body weight

Food consumption and BW gain were slightly reduced in animals receiving 800 mg/kg.

c. Clinical laboratory

APTT was increased in males at  $\geq 400$  mg/kg and in females at all doses (up to 80% in males and 40% in females compared to pretreatment values). Platelet values were missing in many cases due to "technical problems."

Cholesterol and triglyceride values were increased and phosphorus decreased at  $\geq 400$  mg/kg in males (up to ~80, 90, and 15% compared to pretrmt, respectively) and in females at all doses (up to ~100, 100, and 10% compared to pretrmt, respectively).

d. Organ weights and Pathology

There were no organ weight or gross or microscopic pathological changes considered T-R by the sponsor. However, hepatocellular cytoplasmic vacuolization was seen only in treated females (0/2, 1/2, 0/2, and 2/2 in the 0, 200, 400-800, and 600 mg/kg dose groups, respectively), lymphocytic infiltration of the stomach pyloric mucosa was found only in treated males (0/2, 1/2, 2/2, and 1/2) and females (0/2, 2/2, 2/2, and 1/2).

F. ONE YEAR ORAL TOXICITY STUDY OF GP 47680 (SYNTHESIS 2) IN DOGS (Tox ref. 1-26, Report no. T/P (US) 97003 (MIN 961156), conducted by Novartis in 1997, GLP, Vol. 1.45)

1. Methods

Beagle dogs were dosed with 0 (empty gelatine capsules), 60, 200, or 600 mg/kg orally (capsules) for 26 (3/sex/group) or 52 weeks (4/sex/group). An additional 2/sex were included in the C and HD groups for assessment of recovery (4 weeks). Endpoints included clinical observations, toxicokinetics (4/sex/group 0, 2, 4, 6, 8, and 24 hr after dosing on day 1 and during week 6), ophthalmoscopic (predose, weeks 26 and 52) and electrocardiographic (predose and 4-6 hr after dosing during weeks 13, 25, 39, 51, and 56) examinations, clinical laboratory tests (hematology, serum chemistry, urinalysis on all dogs during weeks 13, 26, 39, and 52, and after recovery period), and gross (all animals) and microscopic (all tissues from all non-recovery groups; target tissues from recovery groups) pathology. *Drug Batch #*: 000692

*Dose Justification*: Doses were based on results of 3- and 12-month oral toxicity studies with GP 47680 produced by the old synthetic method and on a 4-week pilot study with Synthesis 2 GP 47680 (above). In the 3-month study (60, 200, 600 mg/kg), emesis occurred at the HD; there were no effects on BW gain; liver wts were increased at all doses; ALT and AST were increased in 1 HD male; hemosiderin deposits were seen in liver Kupfer cells at the MD or greater; and hemosiderin was found in the kidney at the HD. In the 1 year study (60, 200, 600-400 mg/kg), decreased weight gain and thymic atrophy at the HD were the only effects reported. In the 4-week study (200, 400-800, 600 mg/kg), CNS signs and decreased BW gain were reported at  $\geq 600$  mg/kg.

2. Results

a. Mortality and Clinical signs

There were no deaths during the study. T-R signs included ataxia, clonic convulsion, salivation, recumbency and "vomit with feed and/or apparent compound" in HD males and females, and a D-R increase in the incidence of "feces with apparent compound" at all doses. The CNS signs were noted with decreasing frequency only during the first 1/4 of the study period, while the other findings were observed throughout the study.

b. Body weight

There were no T-R effects on food consumption or BW.

c. Ophthalmoscopic examinations

There were no ophthalmological changes that appeared to be related to treatment.

d. Electrocardiographic examinations

There were no T-R ECG changes.

e. Clinical laboratory

PTT was D-D increased in MD and HD males and females during treatment (mean about 20% above C at HD in both sexes). There were no differences between recovery groups.

Cholesterol, triglyceride, and bile acid values were D-D increased at all doses in males (means up to 70, 50, and 50% above C, respectively) and females (means up to 40, 30, and 30% above C, respectively). In addition, phosphorus and potassium tended to be decreased in treated females (up to about 15 and 10% below C, respectively). There were no

differences between recovery groups.

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There were no T-R changes in urinalysis parameters.

f. Organ weights and Pathology

There were no T-R organ weight or gross pathological changes. At 26 weeks, hepatocellular cytoplasmic vacuolization was seen microscopically in females from all dose groups (0/3, 3/3, 2/3, and 3/3 in C, LD, MD, and HD females, respectively), adrenal gland cytoplasmic vacuolization was found in MD and HD males (1/3 and 2/3 in zona glomerulosa compared to 0/3 C) and females (1/3 and 2/3 in zona fasciculata compared to 0/3 C), and pituitary cysts were increased in treated males (0, 1, 1, and 2/3) and females (1, 2, 2, and 2/3). At terminal sacrifice, the incidences of hepatocellular vacuolization were 0/4, 1/4, 2/4, and 1/4 in males and 1/4, 2/4, 1/4, and 3/4 in females from the C, LD, MD, and HD groups, respectively. There were no apparent T-R differences among groups for adrenal and pituitary changes. After recovery, there were no apparent group differences for any organ system examined.

g. Plasma drug levels

The sponsor reported that GP 47680 was not measured due to the instability of the unchanged compound in dog plasma. GP 47779 concentrations were below the level of detection in the majority of samples at all doses; median peak levels at 2 hr post-dose were < 0.3 ug/ml. Despite this reported instability, plasma levels of both parent and metabolite were determined in a more recent 2-week dog study (No. 971059) which examined similar doses of an 'extrafine' batch (400697) of synthesis 2d GP 47680, which had the highest levels of the impurities that result from the new synthesis and is milled to produce a smaller, more uniform particle size (Table III.F.1). The toxicity profile was generally similar to that in other studies of GP 47680. Findings included CNS signs, decreased BW, decreased fibrinogen and reticulocytes, increased cholesterol and triglycerides, increased liver weights, gross discoloration and enlargement of the liver, and vacuolation and centrilobular hepatocyte hypertrophy in the liver, all seen primarily in HD dogs. In addition, decreased HR, P-R interval prolongation, and "grade II" AV block were recorded in some HD males. These were considered of uncertain relation to treatment. CV effects had not been seen previously.

**Table III.F.1** Mean plasma levels of GP 47680 and GP 47779 after oral administration of GP 47680 to dogs (2-3/sex/group) in a 2-week toxicity study

| GP 47680<br>Dose (mg/kg) | Sex | Cmax<br>(ug/ml) |        |          |        |
|--------------------------|-----|-----------------|--------|----------|--------|
|                          |     | GP 47680        |        | GP 47779 |        |
|                          |     | Day 1           | Day 14 | Day 1    | Day 14 |
| 100                      | M   | 3.84            | 6.92   | 0.121    | 0.236  |
|                          | F   | 4.24            | 4.86   | 0.139    | 0.107  |
| 300                      | M   | 9.84            | 8.62   | 0.338    | 0.198  |
|                          | F   | 3.39            | 6.47   | 0.197    | 0.144  |
| 600                      | M   | 4.05            | 10.9   | 0.399    | 0.336  |
|                          | F   | 5.14            | 12.9   | 0.445    | 0.355  |

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G. 3-MONTH ORAL TOXICITY STUDY OF GP 47779 (SYNTHESIS 1) IN DOGS (Tox ref. 2-4, Report no. 19/78/S.L., conducted by [redacted] report dated 1978, no GLP statement, Vol. 1.51)

1. Methods

Beagle dogs (3/sex/group) were dosed with 0 (2% aqueous CMC), 60, 200, or 600 mg/kg orally (capsules) for at least 3 months. Because of excessive toxicity, the HD was reduced to 400 mg/kg on day 13. A second MD group was started 1 month after the start of the main study and dose level was gradually increased over 7 days to reach 200 mg/kg, which was continued for a full 3 months. The original group receiving 200 mg/kg was retained for 4-weeks at the of the 3-month treatment to assess recovery. Endpoints included clinical observations and BW measurements, ophthalmic and hearing examinations, clinical laboratory tests (hematology, serum chemistry, and urinalysis on all dogs pretest, and during weeks 5, 9, 13; week 15 600-400 mg/kg group; week 17 recovery group), organ weights, and gross and microscopic pathology (all tissues in all animals).

*Drug Batch #: Mg 1177*

*Dose Justification:* None given. In an acute oral study of GP 47779 (synthesis 1) in dogs (30, 100, 300, or 1000 mg/kg), the clinical observations included: enophthalmos and hyperemia of the external mucosa and skin at  $\geq 100$  mg/kg; emesis, decreased activity, tremors, and hypertonia followed by hypotonia at  $\geq 300$  mg/kg; and ataxia, tachycardia, recumbency, and salivation at 1000 mg/kg.

2. Results

a. Mortality and Clinical signs

There were no deaths during the study. Clinical signs included tremors at  $\geq 60$  mg/kg; lethargy, ataxia, and vomiting at  $\geq 200$ ; and salivation at 600-400 mg/kg. Because of the severity of signs (ataxia and vomiting) at 600 mg/kg, the dose was reduced to 400 mg/kg, and administration was interrupted in 3 dogs in this group on several occasions (group received 92 doses at 400 mg/kg).

b. Body weight

Food consumption and BW gain were reduced in animals receiving  $\geq 200$  mg/kg. At the end of treatment, mean BW was 38 and 36% below C in HD males and females, respectively.

c. Clinical laboratory

Erythrocyte parameters were decreased at  $\geq 200$ , and the effect was severe in some individuals. Reticulocytes were generally increased. The hematology report on female #402 receiving 400 mg/kg (HGB 5.3%, RBC  $1.62 \times 10^6/\text{cmm}$ ) noted "marked macrocytic anemia with polychromasia and some hypochromasia. Reticulocytes are very high at 36%." Female #502 receiving 200 mg/kg also had severe anemia and a peripheral blood film that showed "polychromasia and hypochromasia with a few macrocytes and nucleated cells." A high proportion of spherocytes and a reticulocyte response (22% at 11 weeks) were also seen in this animal. Red cell parameters remained low in the recovery group animals.

Sodium was increased somewhat and potassium decreased in 2 HD (M & F) animals. There were no other apparent changes in main study or recovery groups.

There were no remarkable urinalysis findings. Urine volume was not measured.

d. Ophthalmic and Hearing examinations

A "sluggish" hearing response in 3 HD and 1 MD dogs was attributed to drug-induced

sedation. Conjunctivitis and keratitis seen in 2 HD dogs was thought to be mechanical in origin, secondary to drug-induced ataxia; and a loss in definition in 1 HD and 1 MD dog was considered secondary to severe anemia in these animals (#s 402 and 502).

e. Organ weights and Pathology

Heart weights (absolute and relative to brain) were decreased at the HD (both -40%), and liver weights (relative to BW) were increased at all doses (40-50% at HD) compared to C. Gross observations of thymic atrophy, enlarged spleen, decreased body fat, and distended gall bladder were reported in HD animals. Yellow-brown pigment (Perle's -) deposits and/or increased incidences of vacuoles were found in the proximal tubules of the kidneys in HD dogs (M #403, F #s 402 & 404), and bile accumulation and "a centrilobular cellular reaction consisting primarily of mononuclear cells with some hepatocyte loss" was described in the liver of one of these animals (F 402). In addition, a marked reduction in spermatogenesis and increased myeloid activity in the bone marrow were noted in 1 HD male (#405). Increased hemosiderin deposition in the liver, kidneys, and spleen and increased extramedullary hematopoiesis were observed at the MD and HD. There were no pathology findings considered T-R in the recovery group.

H. ONE YEAR ORAL TOXICITY STUDY OF GP 47779 (SYNTHESIS 1) IN DOGS (Tox ref. 2-5, Test no. 79-5300, conducted by [REDACTED] report dated 1981, GLP, Vol. 1.52)

1. Methods

Beagle dogs were dosed with 0, 30, 100, or 300 mg/kg orally (capsules) for 26 (2/sex/group) or 52 weeks (4/sex/group). An additional 2/sex/grp were included for assessment of recovery (4 weeks). Because of the severity of clinical signs of toxicity and weight loss, the HD was reduced to 200 mg/kg beginning on week 5. Endpoints included clinical observations, ophthalmoscopic and electrocardiographic examinations (all dogs; pretreatment, weeks 26, 52 and 56), clinical laboratory tests (hematology, serum chemistry, urinalysis on all dogs during weeks -2, 13, 26, 52 and 56), and gross and microscopic pathology (all animals).

*Drug Lot #: 000692*

*Dose Justification:* None provided. A poorly reported, non-GLP study of GP 47779 in dogs (3/sex/grp) administered oral (capsule) doses of 60; 200; and 600-400 mg/kg (HD reduced on day 13 due to signs and BW loss) for 3 months. Decreased BWs and clinical signs consisting of lethargy, ataxia, tremors, salivation, and vomiting were observed at the MD and HD. RBC parameters were decreased in MD and HD animals (anemia marked in some cases), and electrolyte disturbances (increased Na, decreased K) were seen in HD males and females. At necropsy, heart weights were significantly decreased at the HD and relative liver weights were increased at all doses in both sexes. On histopathological examination, hemosiderin deposition (liver, kidney, and spleen) and extramedullary hematopoiesis were found at the MD and HD, and kidney (pigment and vacuoles in the proximal tubules) and liver changes (bile accumulation, mononuclear inflammatory cell reaction, hepatocyte loss) were seen in HD animals. There were no remarkable findings in recovery groups (4 weeks).

2. Results

a. Mortality and Clinical signs

There were 3 deaths during the study, 1 from each dose group (LD F during week 54, MD M during week 41, and HD M sacrificed moribund during week 5. The LD and HD deaths were attributed to systemic infections; no cause of death was identified for the MD dog. T-R signs were noted at all doses in both sexes; these included emesis, salivation, ataxia,

tremors, decreased activity, opisthotonos, and recumbency/prostration. Their severity at the HD, combined with anorexia and dehydration, led to a dose reduction beginning on week 5.

b. Body weight

BW loss occurred in HD males and females, and decreased BW gain was seen in MD females during the first 13 weeks of the study. Much of these differences were subsequently made up, but HD females continued to gain less than C throughout the study. Food consumption was also decreased in these groups compared to C during the treatment period. HD animals gained more than C during the recovery period.

c. Ophthalmoscopic examinations

There were no ophthalmological changes that appeared to be related to treatment.

d. Electrocardiographic examinations

The only significant change in ECG parameters was an increase in mean P-R interval in HD females at week 26. According to the report, this was "of little biological significance."

e. Clinical laboratory

RBC parameters were decreased somewhat (~10%) in HD males and females, while reticulocytes tended to be decreased in treated dogs from all dose groups compared to C and/or pretreatment values (up to 70%; SS in HD males at 52 weeks). There were too few recovery animals to make an assessment.

Sodium was increased in all treated dogs at various intervals (SS in HD males at 13 weeks). ALP was slightly but consistently increased in treated males (up to 50%) and females (up to 90%) at all doses compared to C (but not compared to pretreatment). BUN was increased in HD males compared to C and pretreatment values (~30%). ALP remained elevated in HD males and MD and HD females after recovery.

There were no T-R changes in urinalysis parameters.

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f. Organ weights and Pathology

Liver weights (absolute and relative to BW) were D-D increased in males (rel. wt. up to 30% above C at HD) and females (rel. wt. up to 50%; SS at MD and HD at 52 weeks). These remained elevated in HD males and MD and HD females after recovery.

No gross necropsy findings or histopathologic alterations were considered T-R by the contract lab. However, the incidence of mononuclear inflammatory cell aggregates in the liver was D-D increased in males at 52 weeks: 1/4, 2/4, 4/4, and 4/4 in the C, LD, MD and HD groups, respectively. The HD male that was sacrificed moribund during week 5 was found to have suppurative pericarditis and cytoplasmic vacuolization and sinusoidal ectasia of the liver.

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#### IV. CARCINOGENICITY

A. TWO YEAR CARCINOGENICITY STUDY OF GP 47680 (SYNTHESIS 2) IN MICE (Ref 1-21, Project no. 82-0939, conducted by [redacted] report issued 10/30/87, GLP, Vols. 1.34-1.37)

##### 1. Methods

Mice (80/sex/group) were given doses of 0, 10, 40, 70, or 100 mg/kg in the diet for 24 months. Of these, 50/sex/grp were used for evaluation of carcinogenicity, 10/sex/grp for hematology and clinical chemistry, and 20/sex/grp for blood level determinations and interim sacrifice (5/sex/grp) at 6, 12, 18, and 24 months.

*Strain:* Tif:MAGf (SPF)

*Drug Batch #:* P-Los 800482

*Dose selection:* Doses were selected on the basis of a 3-month dose range-finding study (Project no. 82-0315; Vol. 1.24) in which (calculated) doses of 0, 95, 255, and 897 mg/kg in males and 111, 321, and 1149 mg/kg in females were administered in the diet. None of these doses produced any significant systemic toxicity, and there were no T-R deaths or effects on BW gain. Clinical chemistry changes (increased liver enzymes, cholesterol, and total protein), increased liver weights, and increased incidences of hepatocellular hypertrophy and hepatocyte necrosis were seen primarily at the MD and HD groups, although some minimal hypertrophy and necrosis was seen in 2/5 LD males. Based these liver effects, 100 mg/kg was chosen as the HD for the 2-year study.

##### 2. Results

###### a. Mortality

Mortality rates were comparable among groups with the exception of an increase seen in HD males during weeks 71 - 74 (Figure IVA.1). During this period 18 HD males died compared to 4 C males, and 10/18 HD males that died were found to have myocardial hemorrhage and necrosis when examined histopathologically. There were no differences in survival rates thereafter, and myocardial lesions were not found in any HD males that died after week 74.

Figure IVA.1: Mortality in 2-Year Mouse Carcinogenicity Study

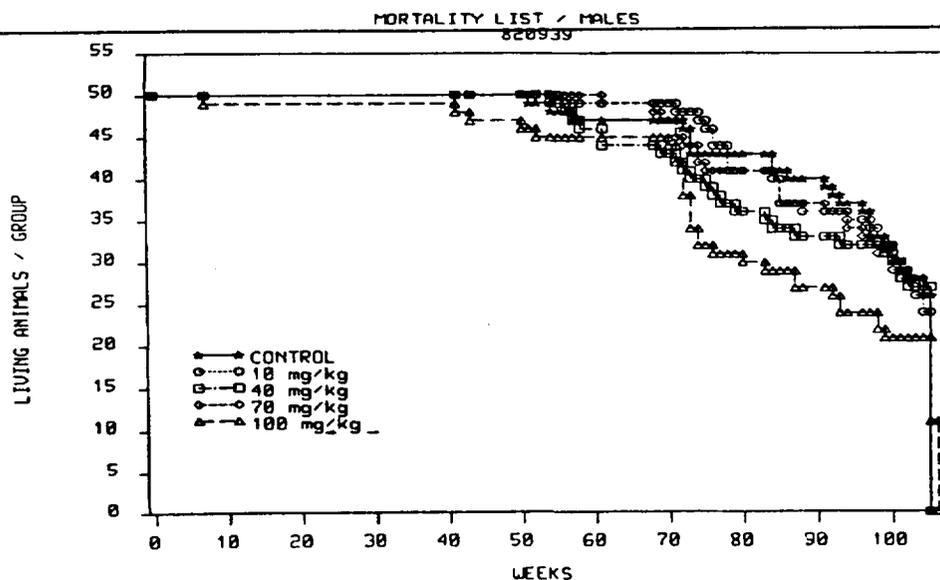
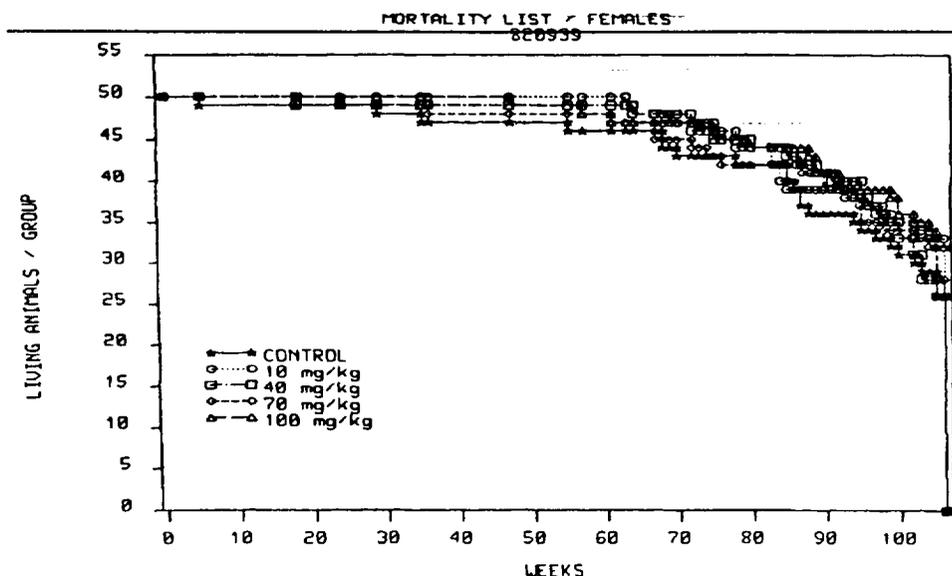


Figure IVA.1 (cont.): Mortality in 2-Year Mouse Carcinogenicity Study



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b. Observed Signs

There were no treatment-related differences in clinical signs.

c. Body Weight and Food Consumption (ad libitum feeding)

Mean BWs over the course of the study are shown in **Figure IVA.2**. After about 70 weeks, means were lower in MHD and HD males compared to C, reaching statistical significance for group MHD males at 74 weeks (5% below C) and for HD males at 70, 74, 82, 89 weeks (5-10% below C). At the end of the study, there were no differences among groups. Means were significantly lower in all treated females compared to C at various times after about 15 weeks, but there were no between group differences among treated females. At 104 weeks, mean BW was 2, 11 (SS), 9, and 13% (SS) below C in LD, MD, MHD, and HD females. The maximum effect over the course of the study was somewhat greater in females (15%) than in males (10%). There was no consistent effect on food consumption.

d. Ophthalmoscopy (performed on C and HD at 6, 12, 18, and 24 months)

Eye examinations did not reveal any evidence of a treatment effect.

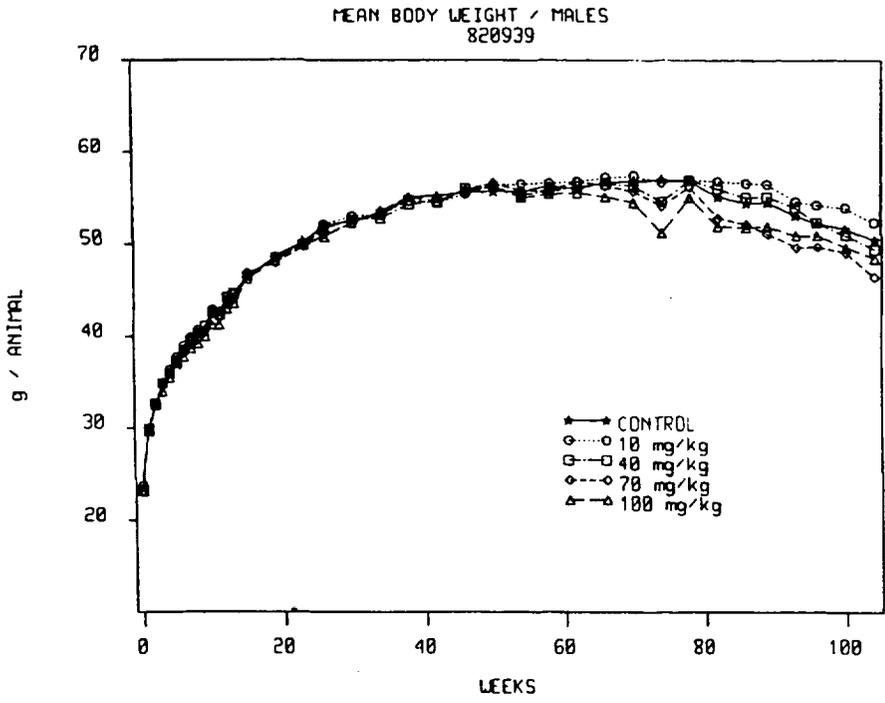
e. Hematology (performed at 14, 27, 53, 79, and 104/105 weeks)

There were no consistent changes in hematological parameters.

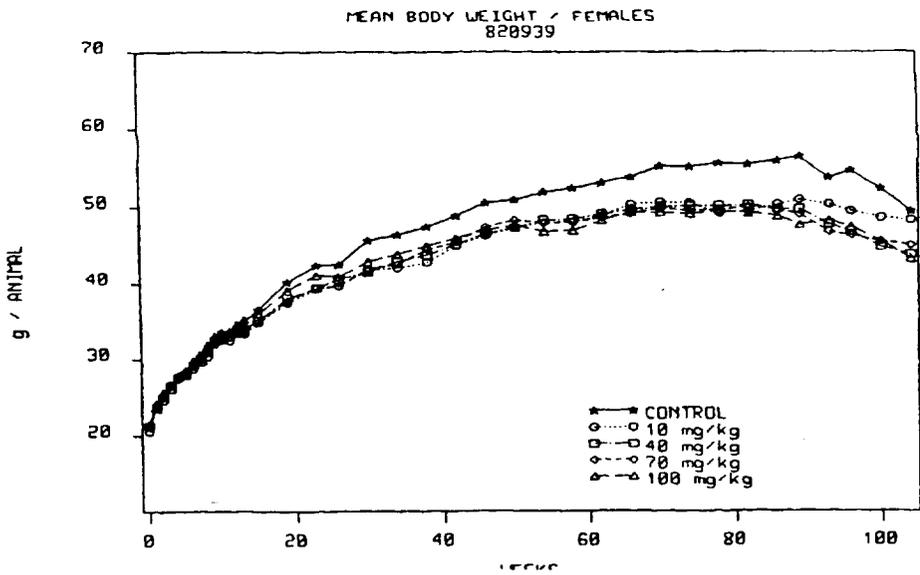
f. Clinical Chemistry (performed at 27, 53, 79, and 104 weeks)

AST, ALT and ALP were elevated in treated males (up to 5, 10, and 3-fold, respectively) and females (up to 2, 4, and 3-fold, respectively) compared to C at various intervals. All groups were affected, although the effects were minimal at the LD. The response was generally dose and time dependent. In addition, glucose levels were increased in treated males compared to C throughout most of the study.

Figure IVA.2: Body Weight in 2-Year Mouse Carcinogenicity Study



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g. Gross Pathology and Organ Weights (organ weights recorded for all animals)

Absolute and relative liver weights were significantly increased in males (up to 2.3-fold C) and females (up to 1.6-fold C) from the 3 highest dose groups. Adrenal weights were also increased in these groups (up to 1.5-fold C). Upon gross examination, enlarged livers and an increased numbers of liver masses were reported in treated males (all doses) and females (top 2 doses).

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

i. Non-neoplastic

Myocardial necrosis in association with myocardial hemorrhage was found in 11/80 HD and 2/80 MHD males. This combined finding was not seen in females or in lower dose group or control males. All animals with this lesion died prematurely (between 362 and 515 days; **Table IVA.1**). Other D-R or HD findings included liver fibrosis (3/80 vs 0 C) and nodular hyperplasia (3/80 vs 0 C) in HD males and fatty atrophy of the exocrine pancreas in MD and HD males (2/80 and 3/80 vs 0 C).

ii. Neoplastic

Benign hepatomas were seen with increased incidences and/or decreased latencies in treated males ( $\geq 70$  mg/kg) and females (100 mg/kg); however, incidences of carcinoma were not different among groups (**Table IVA.2**). There were no other T-R tumor findings.

**Table IVA.1:** Mortality and occurrence of myocardial necrosis associated with hemorrhage in male mice

| Days on Study | Dosage groups - mg/kg |      |      |      |       |
|---------------|-----------------------|------|------|------|-------|
|               | 0                     | 10   | 40   | 70   | 100   |
| 1-356         | 0/8                   | 0/6  | 0/6  | 0/5  | 0/10  |
| 360-369       | 0/4                   | 0/4  | 0/4  | 0/6  | 1/5   |
| 370-489       | 0/5                   | 0/3  | 0/9  | 0/5  | 0/2   |
| 490-519       | 0/4                   | 0/3  | 0/5  | 2/6  | 10/18 |
| 520-736       | 0/59                  | 0/64 | 0/56 | 0/58 | 0/45  |

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**Table IVA.2** Summary of liver findings in mouse carcinogenicity study

| Dose (mg/kg)             | 0  |    | 10 |    | 40 |    | 70 |    | 100 |    |
|--------------------------|----|----|----|----|----|----|----|----|-----|----|
|                          | M  | F  | M  | F  | M  | F  | M  | F  | M   | F  |
| N                        | 80 | 80 | 80 | 80 | 80 | 79 | 80 | 79 | 80  | 79 |
| Liver                    |    |    |    |    |    |    |    |    |     |    |
| nodular hyperplasia      | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 3   | 0  |
| benign hepatoma          | 25 | 13 | 24 | 10 | 29 | 10 | 36 | 14 | 35  | 21 |
| hepatocellular carcinoma | 5  | 3  | 3  | 0  | 3  | 1  | 5  | 2  | 2   | 2  |
| hepatoblastoma           | 1  | 0  | 2  | 0  | 0  | 0  | 0  | 0  | 0   | 0  |

i. Toxicokinetics

Mean plasma levels of GP 47680 in animals sacrificed at 6, 12, 18, and 24 months were 0.15, 0.27, 0.57, and 0.61 ug/ml in males and 0.07, 0.30, 0.37, and 0.63 ug/ml in females at 10, 40, 70, and 100 mg/kg, respectively. Concentrations of GP 47779 were below the limit of detection (0.05 ug/ml) in all but 27 of 147 samples.

B. TWO YEAR CARCINOGENICITY STUDY OF GP 47680 (SYNTHESIS 1) IN RATS (Ref 1-19, Report No. 64-81, conducted by [redacted] issued 7/82, GLP, Vol. 1.30-1.32)

1. Methods

Rats (60/sex/group) were dosed with 0, 25, 75, or 250 mg/kg in the diet for 2 years. Endpoints included standard clinical observations, BW and food consumption measurements, clinical pathology (limited analyses at 13, 26, 52, 78, and 104 weeks on 10/sex/grp), ophthalmoscopic exams (52 and 104 weeks), and gross (complete necropsy performed on all rats; only liver, kidneys, and adrenals were weighed) and microscopic (complete examinations performed on all C and HD animals; liver sections, gross lesions, and tissue masses examined from all animals) pathology exams.

*Strain:* Sprague-Dawley CD

*Drug Lot #:* B78-8, CDF 1935 and B78-8, CDF 2027

*Dose Selection:* In a 6-month dietary study of GP 47680 (Synthesis 1; 100, 300, 1000 mg/kg/day) in rats, treatment-related effects included decreased BW in HD males and in females from all dose groups, increases in BUN and ALT at all doses, increased liver and kidney weights at all doses, and histopathological evidence of changes in the liver (hypertrophy, vacuolar degeneration, nuclear pyknosis) and kidney (cortical tubular alterations and glomerular fibrosis) at  $\geq 300$  mg/kg. There was no treatment-related increase in mortality.

2. Results

a. Mortality

Survival was comparable among groups (**Table IVB.1**).

**Table IVB.1:** Mortality in 2-Year Rat Carcinogenicity Study

| Sex Group         | Male |    |    |    | Female |    |    |    |
|-------------------|------|----|----|----|--------|----|----|----|
|                   | 1    | 2  | 3  | 4  | 1      | 2  | 3  | 4  |
| Died during study | 17   | 17 | 21 | 17 | 31     | 24 | 25 | 21 |
| Survivors         | 43   | 44 | 39 | 43 | 29     | 36 | 36 | 39 |

b. Observed Signs

There were no T-R differences in frequencies of clinical signs.

c. Body Weight and Food Consumption (ad libitum feeding)

Overall BW gain was 8, 10, and 22% lower in males and 8, 30, and 41% lower in females at the LD, MD, and HD, respectively, compared to C. BWs were significantly lower in HD males and in MD and HD females compared to C at weeks 13, 26, and 52, remaining lower in these group to the end of the study (mean BWs 15, 15, and 26% below C at termination, in HDM, MDF, and HDF, respectively; **Table IVB.2**; statistical analysis not performed at week 104). Food consumption was similar among groups.

**Table IVB.2: Body Weights in 2-Year Rat Carcinogenicity Study**

| Dose (mg/kg) | Group Mean Weight in Grams |     |         |      |         |      |          |     |
|--------------|----------------------------|-----|---------|------|---------|------|----------|-----|
|              | Week 0                     |     | Week 26 |      | Week 52 |      | Week 104 |     |
|              | M                          | F   | M       | F    | M       | F    | M        | F   |
| 0            | 206                        | 153 | 581     | 311  | 632     | 354  | 622      | 426 |
| 25           | 203                        | 153 | 572     | 308  | 618     | 349  | 586      | 406 |
| 75           | 205                        | 151 | 574     | 288* | 617     | 311* | 580      | 361 |
| 250          | 206                        | 154 | 550*    | 273* | 587*    | 289* | 530      | 315 |

\* p<0.05

d. Ophthalmologic Examinations

No T-R ocular changes were observed at 52 weeks, but ocular lesions were increased in treated animals (mainly males) from all dose groups at the end of the study, although not always in a strictly dose-related manner (Table IVB.3).

**Table IVB.3 Summary of Ophthalmological Findings (sponsor's table)**

| OPHTHALMOLOGIC FINDINGS   | Dose Level (mg/kg): | Week 104 |        |         |         |          |         |         |         |          |
|---|---------------------|----------|--------|---------|---------|----------|---------|---------|---------|----------|
|   |                     | Group:   | Males  |         |         |          | Females |         |         |          |
|   |                     |          | 1<br>0 | 2<br>25 | 3<br>75 | 4<br>250 | 1<br>0  | 2<br>25 | 3<br>75 | 4<br>250 |
| Number of animals examined  |                     | 44       | 44     | 40      | 45      | 29       | 37      | 38      | 39      |          |
| Number of animals with no ocular lesions  |                     | 40       | 29     | 28      | 28      | 22       | 31      | 29      | 33      |          |
| Eye missing - unilateral  |                     |          |        |         |         |          |         |         | 1       |          |
| Cataracts - unilateral or bilateral; dense, nuclear, and/or lenticular                          |                     |          |        | 1       | 1       |          | 1       |         |         |          |
| Lenticular suture cataract(s) - unilateral or bilateral   |                     |          | 3      |         | 4       | 1        | 3       | 3       | 3       |          |
| Capsular cataract - unilateral or bilateral; focal, posterior, anterior, lenticular, or central |                     |          | 5      | 5       | 7       | 1        | 1       | 3       | 1       |          |
| Corneal abrasion - unilateral or bilateral; nasal, inferior, central or epithelial              |                     |          | 2      | 4       | 1       | 1        | 1       | 1       | 1       |          |
| Corneal hyperplasia - unilateral, epithelial, central   | 1                   |          |        | 1       |         | 1        |         |         |         |          |
| Corneal ulcer - unilateral  |                     |          | 1      |         |         |          |         |         |         |          |
| Corneal neovascularization - unilateral or bilateral; nasal, peripheral, inferior               | 2                   |          | 1      | 3       | 4       | 1        |         | 1       |         |          |
| Retinochoroidal degeneration - unilateral   |                     |          | 1      |         |         |          |         |         |         |          |
| Uveitis - unilateral, nasal, anterior   |                     |          |        |         |         | 1        |         |         |         |          |
| Keratitis - unilateral or bilateral; mild and/or sicca  |                     |          | 5      | 3       | 7       |          | 2       | 1       | 1       |          |
| Iritis - unilateral   |                     |          |        | 1       | 2       |          |         |         |         |          |
| Synechia - unilateral or bilateral; anterior or posterior                                       | 2                   |          | 1      | 1       |         |          |         | 1       |         |          |
| Increased lenticular opacity - unilateral, posterior  |                     |          |        |         |         | 2        |         |         |         |          |
| Pale fundi - bilateral  |                     |          |        | 1       |         |          |         |         |         |          |
| Fibrinoid mass - unilateral, vitreal  |                     |          |        | 1       |         |          |         |         |         |          |

e. Clinical Pathology

Erythrocyte parameters tended to be decreased and reticulocytes increased in treated animals at various times during the study (sometimes SS at MD and HD), although the effect was inconsistent and not always D-R. ALT and/or AST values were decreased in MD and HD males (SS) and females (NS) at several intervals.

f. Gross Pathology and Organ Weights

There were no apparent T-R differences in gross observations.

Kidney and liver weights were increased in MD and HD males (rel wts: 29 and 55% above C, respectively, at HD) and females (rel wts: both 60% above C at HD).

g. Microscopic Pathology

i. Non-neoplastic

A variety of liver changes were increased in incidence and severity in drug-treated animals, both at terminal sacrifice and in those that died or were sacrificed moribund. T-R findings included hepatic centrilobular megalocytosis, vacuolar degeneration of hepatocytes, and cystic degeneration of hepatocytes (Table IVB.4). Chronic progressive nephropathy and various associated renal lesions (mononuclear infiltration, proteinaceous casts, microcalculi, and transitional epithelial hyperplasia of the renal pelvis) were dose-dependently increased in incidence and severity in both males and females from all treatment groups (SS in HD males and females). Focal hyperplasia of the pituitary and pituitary cysts were increased in frequency in treated males. Interstitial cell hyperplasia of the testes was seen with somewhat greater frequency in HD males.

ii. Neoplastic

There was no evidence of a T-R effect on the frequency of neoplasms in animals that died or were sacrificed moribund. At terminal sacrifice, frequencies of hepatocellular carcinoma were D-D increased in treated females compared to C, and incidences of hepatocellular adenoma (hepatic neoplastic nodules) appeared to be increased in treated males (Table IVB.4). Testicular interstitial cell tumors also appeared to be increased in treated males: among survivors, incidences of Leydig cell tumors were 1/43, 2/8, 4/9, and 6/43 in C, LD, MD, and HD males, respectively. When animals that died or were sacrificed prior to 2 years were combined with survivors, incidences were 1/59, 2/13, 4/14, and 8/59, respectively (LD and MD incidences uncertain because of limited nos. of animals examined histologically).

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**Table IVB.4: Neoplastic and Non-neoplastic Liver Findings in 2-Year Rat Carcinogenicity Study of GP 47680**

| Sex                             | Males |      |       |       | Females |       |       |       |
|---------------------------------|-------|------|-------|-------|---------|-------|-------|-------|
| Dose                            | 0     | 25   | 75    | 250   | 0       | 25    | 75    | 250   |
| <u>Vacuolar degeneration</u>    |       |      |       |       |         |       |       |       |
| survivors                       | 0/43  | 1/43 | 1/39  | 6/43  | 1/29    | 5/36  | 3/35  | 3/39  |
| non-survivors                   | 0/17  | 1/17 | 4/21  | 4/17  | 3/31    | 4/24  | 4/25  | 2/21  |
| combined                        | 0/60  | 2/60 | 5/60  | 10/60 | 4/60    | 9/60  | 7/60  | 5/60  |
| <u>Cystic degeneration</u>      |       |      |       |       |         |       |       |       |
| survivors                       | 3/43  | 5/43 | 15/39 | 21/43 | 0/29    | 2/36  | 1/35  | 0/39  |
| non-survivors                   | 1/17  | 0/17 | 5/21  | 3/17  | 0/31    | 3/24  | 3/25  | 0/21  |
| combined                        | 4/60  | 5/60 | 20/60 | 24/60 | 0/60    | 5/60  | 4/60  | 0/60  |
| <u>Megalocytosis</u>            |       |      |       |       |         |       |       |       |
| survivors                       | 0/43  | 7/43 | 24/39 | 33/43 | 3/29    | 10/36 | 20/35 | 39/39 |
| non-survivors                   | 1/17  | 0/17 | 5/21  | 2/17  | 1/31    | 2/24  | 6/25  | 9/21  |
| combined                        | 1/60  | 7/60 | 29/60 | 35/60 | 4/60    | 12/60 | 26/60 | 48/60 |
| <u>Hepatocellular adenoma</u>   |       |      |       |       |         |       |       |       |
| survivors                       | 3/43  | 6/43 | 6/39  | 8/43  | 1/29    | 6/36  | 3/35  | 3/39  |
| non-survivors                   | 2/17  | 1/17 | 2/21  | 1/17  | 1/31    | 1/24  | 3/25  | 0/21  |
| combined                        | 5/60  | 7/60 | 8/60  | 9/60  | 2/60    | 7/60  | 6/60  | 3/60  |
| <u>Hepatocellular carcinoma</u> |       |      |       |       |         |       |       |       |
| survivors                       | 2/43  | 1/43 | 3/39  | 2/43  | 0/29    | 3/36  | 5/35  | 7/39  |
| non-survivors                   | 0/17  | 0/17 | 3/21  | 0/17  | 0/31    | 0/24  | 2/25  | 0/21  |
| combined                        | 2/60  | 1/60 | 6/60  | 2/60  | 0/60    | 3/60  | 7/60  | 7/60  |

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C. TWO YEAR CARCINOGENICITY STUDY OF GP 47779 (SYNTHESIS 2) IN RATS (Ref 2-9, Study No. 951001, conducted by Novartis, issued 4/98, GLP, Vol. 1.55-61)

1. Methods

Rats (60/sex/group) were dosed with 0, 75, 250, or 600 mg/kg by gavage (suspension in 0.5% CMC) for 104 weeks. Parameters included standard clinical observations, BW and food consumption measurements, hematology (no clinical chemistry), ophthalmoscopic exams (C and HD only), and complete gross and microscopic pathology examinations (all animals). Plasma levels of GP 47779 and CGP 10000 (but not GP 47680) were determined in samples collected from 5/sex/group at 2 and 24 hr after dosing during weeks 1, 11, 26, 52, and 78.

*Strain:* Sprague-Dawley CD

*Drug Lot #:* 800394

*Dose Justification:* Dose selection was based on the results of a 13-week oral toxicity study in rats (50, 200, 600, and 2000 mg/kg; previously reviewed). Findings in this study included HD mortality, clinical signs of CNS toxicity (ataxia, hypoactivity), decreased BW gain, hematological changes that indicated effects on RBCs (↓ MCV, MCH, reticulocytes, echinocytes), clinical pathology changes suggestive of effects on the hepatobiliary system (↑ gamma-GT, cholesterol, bile acids, bilirubin) and on renal tubular function (polyuria, proteinuria), increased liver and kidney weights, hepatocellular hypertrophy, and minimal nephropathy (hyaline casts & tubular dilatation). Toxicokinetic data (see **Table IIIC.3**) showed that significant amounts of oxcarbazepine were formed from the metabolite, such that plasma levels of each were similar for a given sex. Levels of both compounds were higher in females than in males. The HD for the 2-year study was lowered from 750 mg/kg on the recommendation of the Division and the CAC.

2. Results

a. Mortality

There was a trend (SS) toward increased survival with increasing dose (**Table IVC.1**).

**Table IVC.1:** Mortality in 2-Year Rat Carcinogenicity Study

| Sex Group         | Male |    |    |    | Female |    |    |    |
|-------------------|------|----|----|----|--------|----|----|----|
|                   | 1    | 2  | 3  | 4  | 1      | 2  | 3  | 4  |
| Died during study | 38   | 33 | 36 | 24 | 43     | 36 | 29 | 22 |
| Survivors         | 22   | 27 | 24 | 36 | 17     | 24 | 31 | 38 |

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b. Observed Signs

On the first few days of treatment, CNS signs (ataxia, decreased muscle tone, hypoactivity, recumbency, head shaking) were noted in HD males and females. Increased incidences of ataxia and perianal staining continued to be seen throughout the study in HD animals.

c. Body Weight and Food Consumption (ad libitum feeding)

BWs were decreased in HD males and MD and HD females, compared to C, throughout the study (terminal BWs 15, 12, and 36% below C, respectively; **Table IVC.2**). Parallel decreases in food consumption were seen in HD males and females (both SS at some intervals).