DIVISION OF NEUROPHARMACOLOGICAL DRUG PRODUCTS
REVIEW AND EVALUATION OF PHARMACOLOGY & TOXICOLOGY DATA
Original Summary

NDA No.: 20796 **Submission Date:** Jan 2, 1998

Drug: COMTAN™ (entacapone) Tablets (200 mg)

Sponsor: Orion Corp.
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Scotch Plains, NJ 07076

Reviewer: T.D. Steele

Indication: Parkinson's disease (adjunct to L-DOPA)

Pharmacologic Class: Catechol-O-Methyl Transferase (COMT) Inhibitor

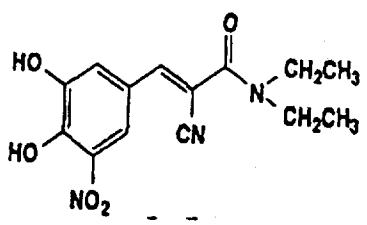
Chemical Information:

CAS Number: 130929-57-6
CAS Name: (E)-2-Cyano-3-(3,4-dihydroxy-5-nitrophenyl)-N,N-diethyl-2-propenamide
IUPAC Name: (E)-2-Cyano-3-(3,4-dihydroxy-5-nitrophenyl)-N,N-diethylacrylamide

Empirical Formula: $C_{14}H_{15}N_3O_5$

Molecular Weight: 305.28

Structure:



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

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A. PHARMACOLOGY

A.1 Mechanism of Action

Entacapone (ENT) inhibits the activity of catechol-O-methyl transferase, the enzyme that catalyzes the methylation of catechols at the 3-hydroxyl position. In this regard, the compound is very similar to tolcapone (TOL, TASMAR™), a recently approved COMT inhibitor. The 3-O-methylation of L-DOPA in the periphery (liver, kidney, gut, plasma), yielding 3-O-methyl-DOPA (3-OMD), is a primary means of L-DOPA inactivation. Thus, COMT inhibition increases plasma exposures to L-DOPA, resulting in higher brain concentrations of L-DOPA and consequently dopamine. The expected net result from the combination therapy with L-DOPA, a peripheral decarboxylase inhibitor (eg. carbidopa) and the COMT inhibitor is more prolonged antiparkinsonian activity. Entacapone appears to poorly penetrate the blood-brain barrier (BBB), so its main effects are purportedly due to peripheral COMT inhibition. TOL also poorly penetrates BBB, but has been suggested to have more significant effects on brain COMT.

The *in vitro* COMT-inhibitory activities of ENT and the Z-isomer were evaluated in crude enzyme preparations from several rat tissues and human RBCs. The following table presents the IC_{50}s (μM) derived in those studies, and compares the values to those obtained for TOL in crude enzyme preparation (data from Pharm/Tox review for TASMAR, NDA 20697):

Tissue	ENT	Z	TOL
rat brain	0.010	0.022	0.010
rat duodenum	0.016	0.034	-
rat RBC	0.026	0.033	-
rat liver	0.160	0.280	0.036
human RBC	0.033	-	-
human recomb.			0.027

The enzyme specificity of ENT was assessed by determining potential inhibitory effects on other enzymes involved in catecholamine metabolism (tyrosine hydroxylase [TH], dopamine-β-hydroxylase [DBH], DOPA decarboxylase [DDC], monoamine oxidase A and B). Measurable, but low, effects were noted only versus TH (IC_{50} = 48 μM). The highest ENT concentration used was 50 μM. TOL was similarly specific. ENT also did not significantly inhibit rat liver phenolsulfotransferase activity.

COMT inhibition was determined in *ex vivo* preparations after treatment of rats with ENT (0.3 - 30 mg/kg, p.o.). The time course of effects of 10 mg/kg ENT (figure from the sponsor's summary on the following page) suggests significant inhibitory effects of ENT lasting 3-5 hrs in peripheral tissues. Striatal COMT was transiently inhibited suggesting that ENT penetrates the CNS to some degree.

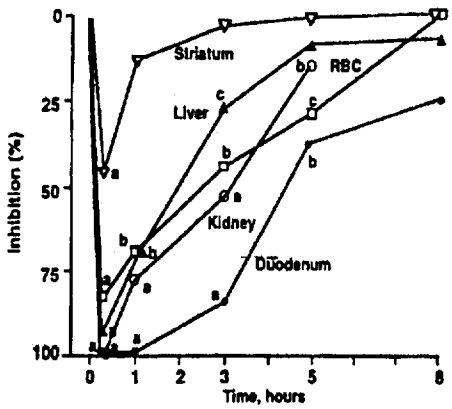

Figure 1. Time response of the effect of entacapone on rat tissue COMT activities. Dose 10 mg/kg p.o. (n=2-6 in control groups and 4-6 in test groups). Statistical significance: a; p<0.001, b; p < 0.01, c; p < 0.05.

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The following table of ED_{50} values (mg/kg) for COMT inhibition by ENT (1 hr post-dose) and TOL in *ex vivo* rat preparations after oral administration indicate that the two compounds are very similar in potency:

	ENT	TOL [b]
duodenum	1.2	0.9
liver	6.7	4.5-6.3
RBC	5.4	4.3
brain	24.2	26-28

[a] data from Review article (Kaakkola et al., Gen. Pharmacol., 25:813, 1994)

A comparative study with ENT and the Z-isomer (10 mg/kg doses) suggested duodenal and liver COMT were equally inhibited by the two isomers, but RBC and brain COMT were inhibited to a lesser degree by the Z-isomer.

An extensive receptor binding screen was not done, but ENT did not show any significant affinity for D1, D2, or α2 receptors.

A.2 Effect of Entacapone on L-DOPA pharmacokinetics

Plasma Levels in Rats

The effect of orally administered ENT (0.3 - 30 mg/kg) on L-DOPA pharmacokinetics was assessed in rats (Han:Wistar; n=5) coadministered L-DOPA and carbidopa (50 mg/kg each).

As expected, ENT caused a dose-dependent reduction in 3-OMD formation, and prolonged the presence of L-DOPA in plasma (Figures 2A and 2B from sponsor). In a subsequent study, 30 mg/kg ENT increased the plasma elimination half-life of L-DOPA by 5-fold after i.v. L-DOPA, and 2-fold after oral L-DOPA.

By comparison, a 30 mg/kg dose of TOL to rats caused an approximate two-fold increase in plasma L-DOPA exposure (from TASMAR NDA). A precise comparison cannot be made because of experimental design differences, but it is noted that the effective doses ranges for the two compounds are similar.

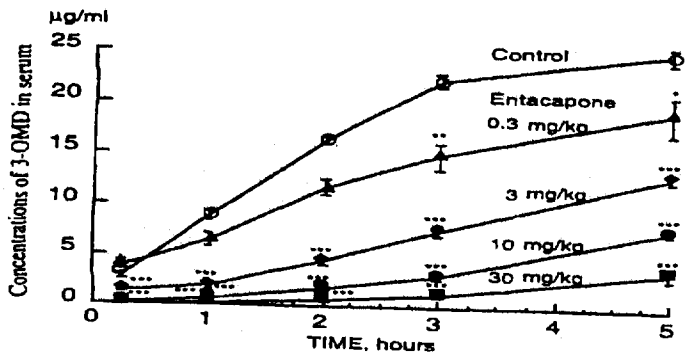

Figure 2A. Dose-and time-related decrease in 3-OMD concentrations by entacapone. Control is levodopa + carbidopa treatment. Statistical significance: *** p<0.001, **p<0.01, *p<0.05 vs. corresponding control; n = 14 for controls and 5 for the treatments.

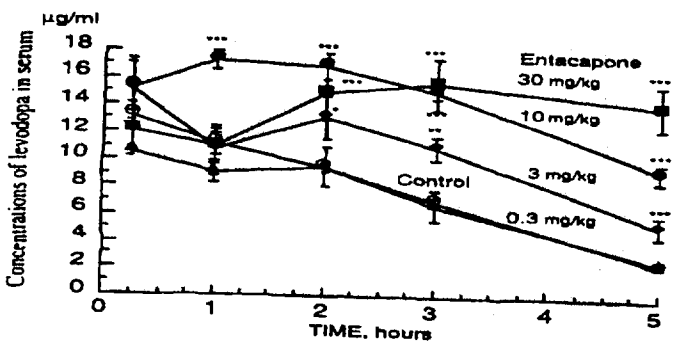

Figure 2B. Long-lasting dose-related levodopa-sparing effect of entacapone. Control is levodopa + carbidopa treatment. Statistical significance: *** p<0.001, **p<0.01, *p<0.05 vs. corresponding control; n= 14 for controls and 5 for the treatments.

Brain L-DOPA and Monoamine Levels in Rats

To assess central COMT inhibitory effects, rats were administered ENT (0.3-30 mg/kg, p.o.) and L-DOPA + carbidopa (50 mg/kg of each), and brains were sampled 2 hr later for analysis of monoamines and metabolites. In a time-course study, 10 mg/kg ENT was administered with L-DOPA + carbidopa (50 mg/kg of each) and brain samples were collected at 1-5 hrs post-treatment.

Entacapone caused dose-related increases in striatal L-DOPA, DA, and DOPAC, and a decrease in 3-O-methyl-DOPA (3-OMD). HVA was unaffected suggesting that central COMT was not inhibited (Sponsor Fig. 4). The time-course study indicated that ENT effects persist for at least 5 hrs, and that HVA is increased by the COMT inhibitor, confirming a peripheral drug effect (Sponsor Fig. 5).

By comparison, a 30 mg/kg dose of TOL increased the striatal L-DOPA AUC by 3-4 times, and decreased striatal HVA (NDA 20697).

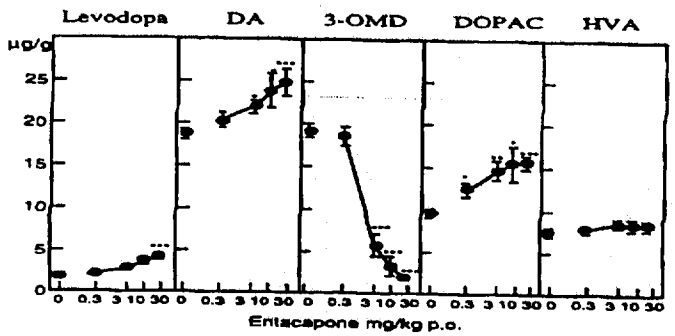

Figure 4. Concentrations of levodopa and its metabolites in rat striatum 2 h after concomitant administration of various doses of entacapone and carbidopa +levodopa (50 + 50 mg/kg). Statistical significance: *** p<0.001, ** p<0.01, * p<0.05, control vs. entacapone treatment, mean ± SE, n = 6.

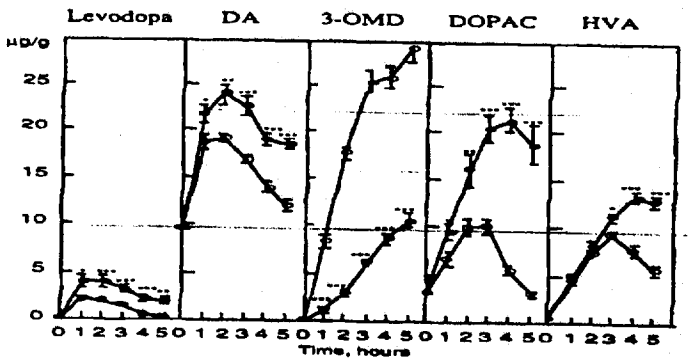

Figure 5. Time-related changes of levodopa and its metabolites in rat striatum after oral levodopa + carbidopa (50 + 50 mg/kg) + entacapone (10 mg/kg) treatment. Statistical significance: *** p<0.001, ** p<0.01, * p<0.05, control vs. entacapone treatment, mean ± SE, n = 6. Open circles(○): levodopa + carbidopa; closed circles (●): levodopa + carbidopa + entacapone.

In a microdialysis study, entacapone doses of 30 and 100 mg/kg, i.p., reduced striatal HVA efflux by >50%, indicating that entacapone inhibits central COMT at high doses.

A.3 Efficacy in Animal PD Models

Reserpinized mice

ENT (10, 30 mg/kg, i.p.) potentiated the effects of L-DOPA + carbidopa (250 mg/kg LD/62.5 mg/kg CD) in reversing hypokinesia in reserpinized mice.

6-OHDA-lesioned rats

Han:Wistar rats were unilaterally-lesioned by injection of 8 µg 6-OHDA into the substantia nigra. After recovery, rotational behavior was tested following administration of L-DOPA (10 mg/kg, p.o.) + carbidopa (10 mg/kg, i.p.), alone or in combination with ENT (1, 3, or 10 mg/kg, p.o.).

The time-course of rotational effects of LD/CD alone and in combination with ENT are in Sponsor's Fig. 13. LD/CD alone caused relatively small rotational activity. Both ENT doses markedly increased the peak effect and duration of action.

A 30 mg/kg, i.p., dose of TOL produced greater rotational activity (up to 240 turns/15 min), but the LD baseline was also higher. The duration of action of TOL was also ~4 hrs (NDA 20697).

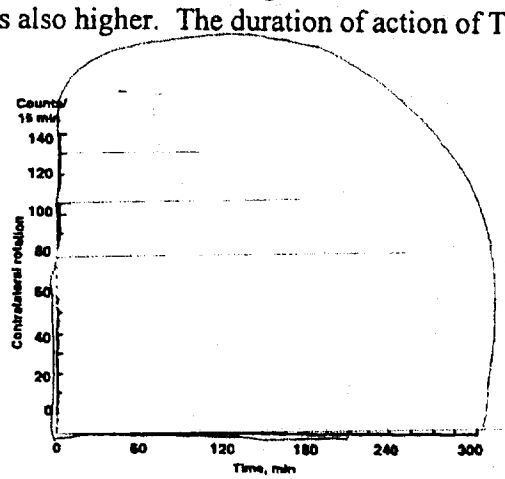

Figure 13. Time-related turnings in 6-OHDA lesioned rats after oral administration of levodopa + carbidopa (10 + 10 mg/kg = control) combined with various doses of entacapone. Statistical significance: $^{*}p < 0.05$, $^{**}p < 0.01$, control vs. entacapone treated, n = 8. Entacapone (1 mg/kg) had no effect (data not shown).

A second study in 6-OHDA-lesioned rats assessed the effects of selegiline in combination with LD/CD and entacapone after repeated (2-week) administration of selegiline. This study also evaluated the development of tolerance to entacapone (2-week treatment). All test groups received LD/CD (10 and 30 mg/kg, p.o). The combination of acute selegiline (2 mg/kg, p.o.) and entacapone (10 mg/kg, p.o.) caused significantly greater and longer rotational response than that caused by either drug alone. Tolerance did not appear to develop to entacapone.

A third study determined that addition of 10 mg/kg ENT allows the LD dose to be lowered by greater than 50% (i.e., the effect of ENT + 5 mg/kg LD was greater than 10 mg/kg LD alone).

MPTP-treated Common Marmosets

This study was conducted at the [redacted]

A total of 16 common marmosets were lesioned with MPTP. The dose and method of administration was not provided, but the lesion appears to be bilateral since locomotor activity and disability scores, and not rotations, were the endpoints. The lesioned animals were divided into 4 groups (n=4), and each group was used for designated sets of experiments.

The total number of experiments was quite large; dose-response data were collected in studies in which the L-DOPA (LD) dose was varied and the entacapone (ENT) dose was varied. The apparent aim of these studies was to determine the regimen that resulted in an optimal response to ENT. LD test doses ranged from 2.5-18 mg/kg (gavage), and ENT dose ranges were from 2.5 - 25 mg/kg. The carbidopa (CD) dose was 12.5 mg/kg and was usually given 30 minutes prior to coadministration of LD and ENT.

In a dose-response study with LD/CD alone (2.5-25 mg/kg), dose-related increases in locomotor activity and improvement of disability scores were observed across the LD dose range. In the dose-response study with ENT (5-25 mg/kg), maximal effects were obtained with 12.5 mg/kg.

The results of experiments with other regimens were variable, and suggest that the response to combination therapy is highly dose-dependent. In most instances, increasing ENT doses to greater than 12.5 mg/kg did not increase the peak effect or duration of response to LD. At high doses of L-DOPA (e.g. 18 mg/kg), the addition of ENT did not substantially increase the peak response but may have slightly increased the duration of action.

The most obvious antiparkinsonian effects of ENT in marmosets occurred when submaximal doses of LD were administered. The sponsor's summary provided the results of experiments (shown below) in which the effects of ENT were clearest. The regimen that produced these results was 2.5 mg/kg LD + 12.5 mg/kg ENT (animals pretreated with 12.5 mg/kg CD). From a clinical standpoint, these studies suggest that patient response to the combination may require careful titration of the individual components, which may not be readily achieved since only one dosage strength (200 mg) of ENT is being developed.

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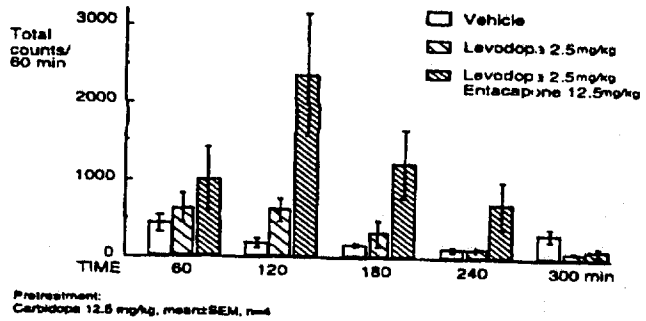

Figure 18. Time related effect of a low dose of levodopa (2.5 mg/kg orally) on locomotor activity in MPTP treated marmosets following pretreatments with 1) carbidopa (12.5 mg/kg orally) or 2) carbidopa + entacapone (12.5 mg/kg of each orally)

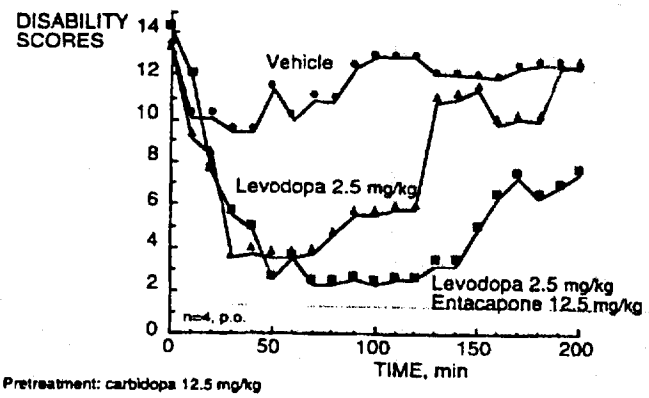

Figure 19. Effect of a low dose of levodopa (2.5 mg/kg orally) on disability scores in MPTP treated marmosets following pretreatments with 1) carbidopa (12.5 mg/kg orally) or 2) carbidopa + entacapone (12.5 mg/kg of each orally).

By comparison, a significant potentiation of rotational response to LD in hemiparkinsonian monkeys was observed after 30 mg/kg TOL orally. A dose of 15 mg/kg was not effective. (NDA 20697)

B. SAFETY PHARMACOLOGY

B.1. Central Nervous System Effects

Oral entacapone doses of 30 and 100 mg/kg were inactive a battery of mouse models for CNS activity (stimulation, motor coordination, anticonvulsant activity, and sedation). A dose of 30 mg/kg slightly potentiated amphetamine-induced stereotypy in rats. Striatal dopamine uptake was weakly inhibited by ENT *in vitro*.

B.2. Cardiovascular/Respiratory Effects

Hemodynamics in Anesthetized Rats

The effect of entacapone on hemodynamics [mean arterial pressure (MAP), systolic arterial pressure (SAP), diastolic arterial pressure (DAP), contractility (dP/dt), heart rate (HR), and electrocardiogram (ECO)] was studied in anesthetized normotensive Han:Wistar male rats. One study assessed the effects of intravenous entacapone alone (0.003 - 3 mg/kg). A second study assessed the effects of pretreatment with oral entacapone (30 mg/kg) on hemodynamic responses to other monoamines.

In the i.v. entacapone study, the four doses of entacapone were given (within animal escalation) at 30 min intervals after a stabilizing period of 40 min. The cardiovascular parameters were registered at 1 min. intervals. No effect on blood pressure, heart rate or ECG was seen with the investigated doses.

In the interaction study, the oral dose of entacapone was given at 10 min before anaesthesia. The cardiovascular responses to injected monoamines were measured 90 min after COMT inhibition in the anesthetized rats. Monoamines (1 and 3 µmol/kg tyramine; 0.25 and 1 nmol/kg isoproterenol; 6 and 12 nmol/kg EPI; 1.6 and 6.4 nmol/kg NE; 0.25 and 1 µmol/kg DA) were given intravenously at 20 min intervals.

No effects on the cardiovascular responses to tyramine, adrenaline or dopamine were observed. Pretreatment with entacapone decreased slightly the positive inotropic effect of noradrenaline while the responses in blood pressure and heart rate were unchanged. According to the sponsor's Results text, entacapone prolonged the cardiovascular effects of ISO, but did not alter the magnitude of the effects.

The sponsor concluded that entacapone has negligible effects on i.v. administered monoamines. This result is somewhat unexpected since entacapone should inhibit their inactivation, and consistently prolong their duration of activity. The fact that entacapone, which has a relatively short half-life, was administered 90 min before the monoamines could account for its lack of effect, and thereby limit the significance and conclusions derived from this study.

In vitro vascular preparations

Potential α-adrenergic contractile effects of entacapone (0.1–30 μM) were assessed *in vitro* in preparations of guinea pig aorta and portal vein. Interactive effects with NE (0.3 μM in aorta), 1 μM in vein) were also evaluated.

Entacapone alone had no effect on vascular tone, nor did it potentiate or block noradrenaline-induced contractions, indicating a lack of α-agonistic or antiadrenergic activity on vascular smooth muscle preparations.

Effect on Respiratory Smooth Muscle

The effect of entacapone (0.2, 1 or 5 mg/kg, i.v.) on bronchial tone (constrictor effect) and histamine-induced bronchoconstriction (dilatory effect) was studied in mechanically-ventilated anesthetized guinea pigs. Entacapone was injected 5 minutes before ascending histamine doses (2 - 32 Mg/kg at 30s intervals). Mean arterial pressure and the heart rate were also recorded. Entacapone did not affect either spontaneous airway tone or histamine-induced bronchoconstriction. No effect on blood pressure or the heart rate was observed.

The effect of entacapone (0.1 - 300 μM) on β2-receptor-mediated relaxation of tracheal smooth muscle was studied *in vitro*. A slight relaxation of intrinsic tone was produced by entacapone (EC_{50} = 40 μM). Entacapone did not relax muscle contracted by carbachol. Entacapone slightly potentiated the relaxing effect of isoproterenol, a substrate for COMT, but not salbutamol.

These studies suggest a reduced potential for bronchial side effects with entacapone.

B.3. Gastrointestinal Effects

Entacapone (10 - 100 mg/kg) did not affect intestinal motility or acid secretion in rat. Mild protection against duodenal ulcers due to cysteamine, and gastric lesions due to aspirin or EtOH, but not indomethacin, was observed. A high concentration (100 μM) partially blocked ACh-induced contraction of the guinea pig ileum.

B.4. Renal Effects

The sponsor reported that 3-100 mg/kg entacapone did not affect diuresis in rat.

A study in the literature (Hansell et al., Acta Physiol Scand, 162:489, 1998) found that entacapone caused a greater than 5-fold increase in sodium excretion. This effect was reduced by pretreatment with the selective dopamine DA1-receptor antagonist SCH23390 suggesting the involvement of DA1 receptor mediated natriuresis involving inhibition of tubular transport processes.

B.5. Effects on Energy Metabolism/Oxidative Phosphorylation

Because of its structural resemblance to 2,4-dinitrophenol, a known uncoupler of mitochondrial oxidative phosphorylation, a series of studies was undertaken to evaluate the potential effects of entacapone on cellular energy metabolism. 2,4-DNP disrupts the proton gradient across the inner mitochondrial membrane, the driving force for ATP generation. The initial effect of uncouplers is a stimulation of mitochondrial respiration and production of heat due to loss of respiratory control. Thus, stimulation of mitochondrial respiration was measured to assess the uncoupling effects of entacapone, DNP, and the structural analog, tolcapone.

In a study with rat liver mitochondria, entacapone appeared to be a relatively weak stimulator of mitochondrial respiration. Surprisingly, tolcapone was more potent than 2,4-DNP:

EC_{50}s for Stimulation of Mitochondrial Respiration

Compound	EC50(μM)
Entacapone	58.0
2,4-DNP	12.5
Tolcapone	2.6

The sponsor speculates that the greater lipid solubility of tolcapone may be responsible for its higher potency.

A second study was conducted in the murine T cell lymphoma cell line L1210 to assess drug effects on mitochondrial respiration at the cellular level. As shown in the following table, cellular respiration (determined from the rate of oxygen consumption in the cultured cells) was not affected by entacapone. Successive (cumulative) additions of entacapone also did not stimulate respiration. However, 2,4-DNP and tolcapone markedly increased the rate of oxygen consumption, possibly due to an uncoupling of oxidative phosphorylation:

Change (%) in Rate of Oxygen Consumption by L1210 Cells

Compound	Conc (μM)			
	5.4	10.9	21.7	43.5
Entacapone	- 7	- 8	+ 3	+ 5
2,4-DNP	n.d.	+ 6	+ 54	+ 97
Tolcapone	+ 46	+ 60	+ 70	n.d.

To assess the potential significance of uncoupling of oxidative phosphorylation at the tissue level, the effects of entacapone (0.3 - 100 μM) were compared to those of tolcapone (0.3 - 10 μM) in the isolated guinea pig heart, the function of which is critically dependent on aerobic ATP synthesis. Functional parameters (heart rate, systolic pressure, end-diastolic pressure, coronary flow, dP/dt), oxygen consumption, and myocardial nucleotide concentrations were measured.

Entacapone reduced systolic pressure and dP/dt, and increased coronary flow, end-diastolic

pressure, and oxygen consumption at concentrations of 30 - 100 μM. Effects of tolcapone were evident at 1 - 10 μM. Concentrations of 10 μM entacapone and 1 μM tolcapone caused equivalent 17% reductions in myocardial ATP content. These data suggest both compounds may uncouple oxidative phosphorylation and impair ATP synthesis, but differ in potency by about 10-fold (TOL > ENT).

A potential manifestation of oxidative phosphorylation uncoupling was assessed at the whole animal level in a study of drug effects on body temperature in rats. An uncoupling of ox-phos with subsequent dissipation of heat should elevate body temperature. In contrast, DA agonists generally reduce body temperature. Single oral doses of 400-800 mg/kg ENT did not affect body temperature, but 2,4-DNP (20 mg/kg, p.o.) and tolcapone (50-400 mg/kg, p.o.) significantly increased body temperature (Sponsor Fig. 20). A second single dose study with 25-50 mg/kg tolcapone showed no significant effects. Combining ENT (400 mg/kg) with LD (10 mg/kg)/CD (20 mg/kg) also did not cause hyperthermia. Repeated dosing with ENT also did not significantly elevate body temperature, but hyperthermic effects of TOL and DNP were evident by days 3-4.

The body temperature increase by TOL and DNP were in the range of 0.5 - 1.0 degree. While statistically significant, these increases are rather small and of questionable toxicological relevance. Further, TOL did not cause any clinical chemistry changes suggestive of toxicity. Plasma concentrations of TOL were approximately twofold higher than those of ENT at equivalent dose levels (200 mg/kg/day)

In a study from the sponsor of TOL, oral TOL doses of 10-100 mg/kg did not affect body temperature in mice (NDA 20697).

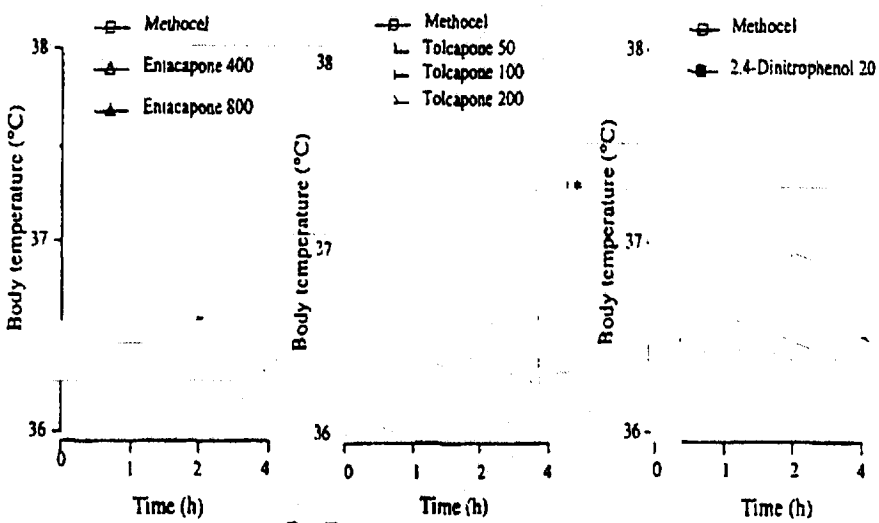

Figure 20. The effect of a single oral dose (mg/kg) of entacapone , tolcapone or DNP on the body temperature of rats (n=10, * $p<0.05$, ** $p<0.01$).

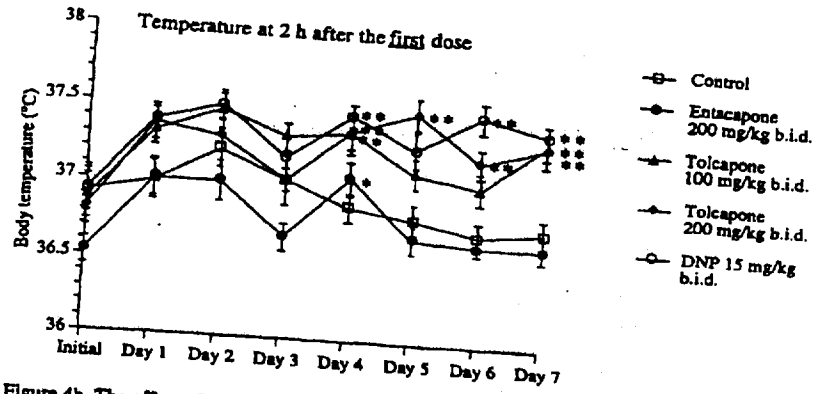

Figure 4b. The effect of repeated oral dosing (b.i.d. for 7 days) of entacapone or tolcapone on the body temperature of rats. The temperature at two hours after the first dosing. Mean±SE, n=8, * p<0.05, ** p<0.01, Dunnett's test for change from the initial value.

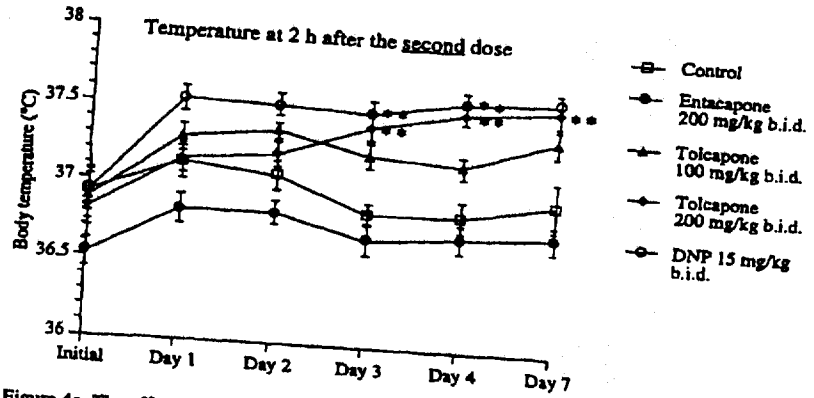

Figure 4c. The effect of repeated oral dosing (b.i.d. for 7 days) of entacapone or tolcapone on the body temperature of rats. The temperature two hours after the second dosing. Mean±SE, n=8, * p<0.05, ** p<0.01, Dunnett's test for change from the initial value.

B.6. Iron(III)-chelation Properties

Since many catechols chelate iron, this potential property of entacapone was evaluated *in vitro*. High concentrations of entacapone (1000 μM) formed a stable complex with Fe^{+3}. Because entacapone may not readily penetrate cell membranes (log D = -0.23), and is extensively glucuronidated, the *in vivo* relevance of this finding is considered questionable by the sponsor. However, dose-related decreases in RBC parameters suggestive of an iron-deficiency anemia, were consistently observed in multiple species in toxicology studies.

B.7 Renal Biochemistry

Two additional biochemical studies were conducted to assess other possible mechanisms, besides α2-μG accumulation, that might be related to the renal changes observed in the chronic toxicology and 2-year bioassays. Entacapone did not decrease the content of nonprotein sulfhydryl groups (mainly glutathione) in kidneys of male or female rats treated with 400 or 600 mg/kg for 14 days. Such a decrease would be expected if reactive (e.g.. electrophilic) compounds were present in the kidneys. Entacapone also did not significantly alter COMT levels in the kidney.

C. TOXICOLOGY

C.1. Acute Toxicology

The following table briefly summarizes the major findings from acute toxicity studies of entacapone alone and in combination with L-DOPA/benserazide. The acute toxicity of the Z-isomer was also assessed. Based on the high lethal doses, transient symptomology, and absence of long-term changes, the acute toxicity of entacapone is regarded as low.

Species (Strain)	Treatment	N	Results
Mice (Crl:NMRI); fasted	1000, 1500, 2000 mg/kg, p.o. ENTAC	5/sex	Mortality: 2MDM, 2 HDM (0-30 min post-dose) Signs: hypoactivity, piloerection, colored urine. No changes evident at 14 days.
Mice (Crl:CD-1); non-fasted	1000, 1500, 2000, 2500 mg/kg, p.o. ENTAC	10 F	Mortality: 2 @ 1500, 4 @ 2000, 3 @ 2500 (0-2 hr post-dose) Signs: hypoactivity, piloerection, tachypnea, dyspnea, ataxia, colored urine. No changes evident at 14 days.
Rats (Wistar)	1500, 1750, 2000 mg/kg, p.o. ENTAC	HD: 5/sex LD, MD: 5F	Mortality: 2 HDF, 1 LDF (0-24 hr post-dose) Signs: hypoactivity, piloerection, colored urine, vocalization. Paresis and convulsions in 1 HDF. No changes evident at 14 days.
Mice (Crl:NMRI)	1000, 1500, 2000 mg/kg, p.o. Z-ISOMER	HD, LD: 5M MD: 5/sex	Mortality: 3 HDM, 1 MDM, 1 MDF (< 1 hr post-dose, except 1 HDM at 5-7 days) Signs: hypoactivity, piloerection, colored urine; convulsions in 1 HDM. No changes evident at 14 days.
Rats (Wistar)	Combo. E/LD/B 1500/1500/375 1250/1250/313 1000/1000/250 0/2000/500	5M	Mortality: 4 HD, 1 MD (0-5 hr 1 hr post-dose) Signs: hypoactivity, piloerection, stereotypy, hunched posture aggression; colored urine in ENTAC animals. No changes evident at 14 days.

C.2. Subchronic Toxicity Studies

C.2.a. Entacapone: 13-Week Dose Range Finding Study in Mice with Administration by Gavage
GLP Research Report #[redacted]7877 Sponsor Volume: 1.12
Conducted by: [redacted]

Summary:

Entacapone (20, 200, 400 mg/kg/day) was orally administered to mice for 13 weeks to determine carcinogenicity study dosages. An additional group received 300 mg/kg/day for 8 weeks, and 600 mg/kg/day for the remainder of the study. The only apparent drug-related effect was a yellowing of skin and cage shavings, the latter likely due to urine coloration. Body weight gain reductions were not dose-related. Toxicokinetic data demonstrated generally dose proportional increases in entacapone exposures between 200-400 mg/kg, but possibly saturated absorption at the highest level. A very limited histopathological analysis of a few tissues revealed no significant findings.

The study provided no useful toxicity information for carcinogenicity study dosage selection, but saturation of absorption may be an acceptable endpoint.

Methods:

Animals:	Crl:CD-1 mice; 6 wks; M: 25-33g, F:20-27g
N:	10/sex/group for main study, 32/sex/group for TK
Doses:	0, 20, 200, 300/600, 400 mg/kg/day (the 300 mg/kg dose was increased to 600 in wk 8 due to lack of toxicity)
Route/Veh:	Oral (gavage) in 0.5% methylcellulose (5 ml/kg)
Lot:	Batch 007 (assay: 101%)

Results:

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Mortality: 3 unscheduled deaths (1 ConM, 1 200F, 1 400F); no cause identified.

Clinical: Yellow staining of skin; orange/red cage shavings (all ENT groups).

Body Wt: A tendency for reduced body wt gain in drug-treated groups was noted early in the study, but recovery over the latter few weeks resulted in no significant difference among groups.

Body Wt Gain, g (% Control)
MALES

	0	20	200	300/600	400
wk 0-7	3.9	3.6 (92)	3.1 (79)	2.4 (62)	1.9 (49)
wk 7-13	1.4	2.0 (143)	2.2 (157)	2.7 (193)	3.4 (243)
wk 0-13	5.3	5.6 (106)	5.3 (100)	5.1 (96)	5.3 (100)

FEMALES

	0	20	200	300/600	400
wk 0-7	4.0	3.9 (98)	3.1 (78)	3.1 (78)	3.1 (78)
wk 7-13	2.5	1.6 (64)	2.7 (108)	3.1 (124)	2.8 (112)
wk 0-13	6.5	5.5 (85)	5.8 (89)	6.2 (95)	5.9 (91)

Food/Water Intake: No treatment-related effects

Clinical Pathology: Parameters were not assessed

Organ Weights: No treatment-related effects

Gross Path: Yellow staining of the skin in all groups was attributed to the color of the drug.

Histopathology: Only the liver, gall bladder, kidney, spleen, stomach, and duodenum were examined. No treatment-related findings were identified.

Toxicokinetics: (3-8 mice/group/time point)

		20		200		300		400		600	
		M	F	M	F	M	F	M	F	M	F
$C_{10\ min}$ (µg/ml)	Day 1	5.0	12.3	28.4	45.6	29.4	67.7	32.2	70.7		
	Day 91	4.3	6.0	24.3	56.4			41.7	53.6	47.3	71.5
$AUC_{0-\infty}$ (µg.h/ml)	Day 1	1.9	4.7	41.4	48.7	52.1	94.9	66.4	105		
	Day 91	1.4	2.6	30.4	72.5			91.9	110	83.0	111

Exposures increased with dose between 20 and 400 mg/kg, but absorption may have been saturated above that level. Exposures were relatively similar on day 1 and 91 indicating that the drug does not accumulate. Levels tended to be higher in females.

C.2.b. 13-Week Oral Toxicity of OR-611 in the Rat

GLP Research Report #: F90062870199 Sponsor Volume: 1.13
Conducted by: Orion Research Center, Espoo, Finland (completed 5/2/91)

Summary:

Entacapone (0, 10, 65, 400 mg/kg/day) was orally administered to rats for 13 weeks to determine dosages for a carcinogenicity study. An MTD for the CA study could be based on a reductions in body weight gain at the HD (13% in both sexes). The only other notable drug-related findings were slight reductions in Hb and Hct in HDM, and yellowing of the fur and urine due to the drug's color. Cytochrome P_{450} content was not increased by entacapone. No major target organ toxicities were observed.

The NOAEL for the study is 65 mg/kg/day.

Methods:

Animals: Crl:CD Sprague-Dawley rats; 6 wks; M: 181-253g, F: 138-190g
N: 12/sex/group
Doses: 0, 10, 65, 400 mg/kg/day
Route/Veh: Oral (gavage) in 1.2% methylcellulose (10 ml/kg)
Lot: Batch 005 [redacted]

Results:

Mortality: 4 unscheduled deaths (1 LDM, 1 MDM, 2 HDF); these were attributed to gavage error or intercurrent pneumonitis, which were reasonably supported.

Clinical: Dose-dependent increase in urine coloration. Yellow staining of fur and tail, and salivation at HD.

Body Wt: Reduced by 13% at the HD in both M and F

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Body Wt Gain (% Control)

	0	10	65	400
Males	364.2 -	374.4 (103)	368.8 (101)	315.2 (87)
Females	167.1 -	168.7 (101)	158.4 (95)	145.5 (87)

Food/Water Intake: No treatment-related effects

Ophthalmoscopy: No treatment-related effects

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Hematol (wk 12): Hct and Hb were slightly reduced in HDM (4-6%)

Clin Chem (wk 12): Abnormal elevations in isolated animals (K in 1 HDM, urea & creatinine in 1 MDM, AST & ALT in 1 HDF) were not associated with any histopathologies.

Mean reductions in blood glucose (HDM, HDF) and triglycerides (HDM) are not considered toxicologically significant.

Urinalysis (wk 12): Hb was present in MD and HD animals of both sexes, and one HDF had a high protein level (100 mg/dl).

Cyto P_{450}/non-protein sulfhydryl content analysis: No changes in P_{450} content were seen. Females showed a dose-related increase in NPS content.

Organ Weights: No treatment-related effects.

Gross Path: Yellow staining of the fur in MD and HD animals due to the color of the drug.

Histopathology: No treatment-related findings were identified.

Toxicokinetics: Plasma concentrations (ng/ml) of entacapone (E) and the Z-izomer were determined 1 hr post-dose during weeks 2 and 11.

		10		65		400	
		M	F	M	F	M	F
Wk 2	E	196	178	5124	3797	14545	15795
	Z	nd	nd	82	48	233	223
Wk 11	E	536	307	14400	9043	37033	52617
	Z	nd	nd	220	97	1023	1122

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ON ORIGINAL

C.2.c. OR-611: 14-Week Oral (Capsule) Subchronic Toxicity Study in the Beagle

GLP Research Report #: 6753-544/23 Sponsor Volumes: 1.15

Conducted by: [redacted]

Summary:

Entacapone (10, 45 and 300 mg/kg/day; final dose achieved by escalation) was orally administered in gelatin capsules to beagle dogs for 13 weeks. Plasma concentrations were determined at a single time point post-dose to demonstrate proof of absorption. The high dose was limited by emesis. Body weight and food consumption were reduced at the HD. A slight, non-significant reduction in RBC parameters in HDF is noted as consistent with hematological changes in other species. No remarkable changes in clinical chemistry, urinalysis, or ECG were observed. A slight, dose-related increase in liver weights was observed, as was an increase in cytoplasmic vacuolation in centrilobular areas of the liver. The vacuole contents were not determined (negative for glycogen).

The NOAEL in this study was 45 mg/kg.

Methods:

Animals:	Beagle dogs;	4-6 mos;	M: 6.1-11.4 kg, F: 6.2-8.9 kg
N:	4/sex/group		
Final Doses:	0, 10, 45, 300 mg/kg/day		

Final doses were acheived after an escalation phase (one wk for LD and MD; 4 wks for HD). Doses were selected based on range-finding and 28-day studies, in which the top dose was limited by emesis.

Route/Veh: Oral (capsule)

Lot: Batch 004[redacted]

Results:

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ON ORIGINAL

Mortality: None

Clinical: Dose-related increase in emesis, dark feces, and colored urine (red to orange).

Body Wt: Significantly reduced at HD (22 and 36% of control in M and F, respectively).

Food Intake: Significantly reduced in MDF, HDF, and HDM.

Ophthalm/EKG/BP (wk 14): No treatment-related effects

Hematol (wks 5, 14): Slight (non-sig) reduction in Hb, Hct, and RBC in HDF (wk 14).

ClinChem (wks 5,14): Isolated abnormal findings were recorded; none were accompanied by

histopathological findings, or appeared related to treatment.

Urinal (wks 5, 14): Some samples from HD animals were darker and had higher specific gravities than control samples.

Organ Wts: D-R (non. sig.)↑ rel. liver wt in HDM (16%) and HDF (20%).

Gross Path: No treatment-related findings.

Histopath: Cytoplasmic vacuolation of centrilobular hepatocytes was seen in 1 MDM, 3 HDM, 1 LDF, and 1 HDF. The vacuoles were not positive for glycogen (PAS stain). The finding appears treatment-related, but is not clearly hepatotoxicity.

Toxicokinetics: Plasma concentrations (μg/ml) were determined at 1 hr post-dose to demonstrate proof of absorption. The data were extremely variable data (SD sometimes greater than means) which limits conclusions on kinetic behavior. The conversion to the Z isomer appeared to be greater than that observed in rats.

		10		45		200		300	
		M	F	M	F	M	F	M	F
Wk 2	E	1.30	0.80	4.26	5.62	13.90	12.74		
	Z	0.34	0.23	1.29	1.59	3.88	3.30		
Wk 8	E	0.33	0.18	3.12	4.26			5.34	19.61
	Z	0.12	0.07	1.01	1.34			1.39	4.48
Wk 11	E	0.60	0.54	6.34	5.33			35.78	14.21
	Z	0.25	0.14	1.54	1.55			8.31	3.45

C.3. Subchronic Combination Studies with Sinemet

C.3.a. The Combination of Entacapone, Levodopa, and Carbidopa: 13-Week Oral Toxicity Study in the Rat

GLP Research Report #: F93061210438 Sponsor Volume: 1.19
Conducted by: Orion Research Center, Espoo, Finland (completed 12/22/93)

Summary:

Potential toxicological interactive effects of entacapone in combination with Sinemet were assessed in rats. Increasing doses of a fixed ratio (4:4:1 E:LD:CD) were administered. Clinical signs of toxicity were evident at ≥ the MD combination, and body weight gain was reduced in males of the HD combination. Some minor urinalysis changes were evident in HD combination animals. The only histopathological change considered treatment-related by the sponsor was focal erosion of the glandular epithelium. Thus, this study did not provide any evidence of any unexpected toxicological interactions with the combination of entacapone and Sinemet.

Toxicokinetic analysis confirmed absorption of entacapone. Entacapone increased L-DOPA, but reduced carbidopa exposures, possibly by interfering with carbidopa absorption.

Methods:

Animals: Crl:CD Sprague-Dawley rats; 6 wks; M: 198-238g, F: 158-196g
N: 10/sex/group
Doses:

mg/kg/day

Group	Entacapone (E)	L-DOPA (LD)	Carbidopa (CD)
1	0	0	0
2	20	20	5
3	50	50	12.5
4	120	120	30
5	120	0	0
6	0	120	30

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No justification or rationale for dose selection or design was provided. It is noted that in a 4-week study (not comprehensively reviewed), a high dose combination of 600/50/50 mg/kg/day E:LD:CD was generally well-tolerated, except for large reductions in body weight gain (20-30%), and produced no evidence of interaction toxicity.

Some dosing solutions were contaminated with carbidopa degradation products upon storage. This is not considered to have significantly impacted the outcome or interpretation of the study.

Route/Veh: Oral (gavage) in 0.5% methylcellulose (5 ml/kg)
Lot: Batch 010[redacted]

Results:

Mortality: 2 unscheduled deaths occurred (1 ConF, 1 G4F); not treatment-related.

Clinical: Clinical signs were evident in groups 3-6. Salivation, colored urine, and stained coat were attributed to E. Salivation, piloerection, flaccidity, and hypoactivity were attributed to LD/CD.

Irregular respiration, high-stepping gait, and paddling were due to the combination (G4 only).

Body Wt Gain: Reduced by 18% in G4M; no effect in any female groups.

Food/Water Intake: Food consumption was reduced 10% in G4M. Water consumption was increased by 30% in G4.

Ophthalm (wk 13): No treatment-related effects (only control and HD animals examined).

Hematol (wk 13): No treatment-related effects.

Clin Chem (wk 13): No toxicologically meaningful treatment-related effects.

Urinalysis (wk 13): Urine volume was decreased, and urinary osmolality and concentrations of Cl and K were increased in G4M. Total urinary excretion of K was increased in G4F.

Organ Weights: No toxicologically meaningful treatment-related effects.

Bone marrow: No changes in the percentages of myeloid and erythroid cells or in the M:E ratio were evident in G4, G5, or G6 animals.

Gross Path: Yellow staining of the fur and tail occurred in all animals treated with ENT. The non-glandular epithelium and pylorus were slightly yellow in some ENT-treated animals (G3, G4, G5).

Red spots on the glandular epithelium were observed at the highest incidence in animals treated with the HD combination (G4: 3M, 4F). Other animals affected were 2 G2M, 1G2F, 1 G5M, 2 G5F, and 1 G6M.

Histopathology: Complete analysis was done only on controls and groups 4, 5, and 6. The stomachs of G2 and G3 animals were also examined.

The only finding considered treatment-related by the sponsor was focal erosion of the glandular epithelium (1 G2F, 2 G4M, 1 G4F, 1 G6M).

Toxicokinetics: Plasma samples were collected during weeks 2 and 11 at 1, 5 and 24 hrs after dosing. AUCs were not determined from these samples, but the following observations were made:

- E levels were two times higher in G5 (ENT only) than in G4 (ENT + LD + CD) at one hr after administration, suggesting the LD/CD may slow ENT absorption. At 5 hrs, levels were comparable in G4 and G5.
- LD levels were higher in G4 versus G6.
- CD levels were lower in G4 than in G6, suggesting that ENT may decrease the absorption of CD.

Levels did not appear significantly different between weeks 2 and 11 (no formal analysis conducted).

A more extensive analysis with frequent sampling (10, 30 min, 1, 2, 3, 4, 5, 6, 8, 10, 12, 24 hrs post-dose) on days 1 and 7 was done in a separate study. The results from day 7 are in the following Table:

Dose E/LD/CD

		20/20/5	50/50/12.5	120/120/30	120/0/0	0/120/30
E	Cmax (μg/ml)	7.6	11	20	27	
	AUC_{0-24} (μg.hr/ml)	9.3	30	71	92	
LD	Cmax (μg/ml)	2.5	4.5	14		8.5
	AUC_{0-24} (μg.hr/ml)	11.0	43	120		85
CD	Cmax (μg/ml)	0.14	0.15	0.45		1.0
	AUC_{0-24} (μg.hr/ml)	0.23	1.8	4.6		20

As expected, entacapone increased exposure to L-DOPA, but decreased exposure to carbidopa. Plasma catecholamine metabolites were altered as expected (↓ 3-OMD, HVA; ↑ DOPAC).

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ON ORIGINAL

C.3.b. Entacapone, Levodopa, and Carbidopa Combination: Toxicity to Cynomolgus Monkeys by Repeated Oral Administration for 13 Weeks

GLP Research Report #: [redacted]68/943243 Sponsor Volume: 1.22
Conducted by: [redacted] Completed: 7/8/94

Summary:

Potential toxicological interactive effects of entacapone administered in combination with Sinemet were assessed in cynomolgus monkeys. Increasing doses of a fixed combination (4:4:1 E:LD:CD) were tested. The major toxicities were overt signs typical of excessive dopaminergic stimulation in the HD combination and HD Sinemet groups. No other toxicologically significant clinical pathology changes or major target organ toxicities were observed.

Peak plasma levels of L-DOPA were lower in animals treated with the HD combination suggesting that E may delay or decrease L-DOPA absorption. Total LD exposure was also not increased by ENT, possibly because ENT inhibited the absorption of carbidopa, allowing more L-DOPA to be converted to DA.

Entacapone exposures in monkeys treated with the HD combination were 3- to 5-fold lower than estimated human exposures at the maximum recommended daily dose (AUC = 15 µg.hr/ml). Monkey L-DOPA exposures after the HD combination were 10-16 times greater than human therapeutic exposures (3 µg.hr/ml).

Methods:

Animals: Cynomolgus monkeys; 18-36 mos; 2.1-3.0 kg
N: 4/sex/group
Doses:

mg/kg/day

Group	Entacapone (E)	L-DOPA (LD)	Carbidopa (CD)
1	0	0	0
2	20	20	5
3	40	40	10
4	80	80	20
5	80	0	0
6	0	80	20

Dose selection was based on a 4-week dose-ranging study (not comprehensively reviewed) in which clear clinical signs were evident at the 80/80/20 (E/LD/CD) mg/kg/day level, and moribund sacrifice was necessary for animals treated with 200/200/50 mg/kg/day (behavioral abnormalities, anorexia, weight loss).

Route/Veh: Oral (gavage) in 0.5% methylcellulose (5 ml/kg)
Lot: Batch 011[redacted]

Results:

Mortality: none

Clinical: Clinical signs were evident in groups 3-6. Stereotypy, akathisia, chewing and agitation were evident with the following order of incidence and severity:

G4 >= G6 > G3 > G5 >= G2

Chorea, dystonia, and uncoordinated behavior were evident mainly in G4 and G6; only isolated incidences occurred in animals of G3 and G5.

Occasional vomiting, salivation, and discolored urine were seen in all treatment group. Dark feces were evident in animals receiving the HD of entacapone (G4 and G6).

Body Wt Gain: No treatment-related effect.

Food Intake: No treatment-related effect.

Ophthalm (wk 13): No treatment-related effects.

ECG (wks 6, 13): No treatment-related effects.

Hematol (wks 6, 13): The following slight, but statistically significant changes were noted; RBC and PCV changes may have been due to abnormal high control levels (increased from pretest measurement):

↓ RBC	-	G4M, G4F (wk 6)
↑ MCHC	-	G5M (wk 13)
↓ PCV	-	G6F (wk 6)
↑ neutros	-	G6F (wk 6)

ClinChem(wk 6, 13): No toxicologically meaningful treatment-related effects.

Urinal. (wk 6, 13): Discolored urine was seen in animals of G3, G4, and G5. Total reducing substances were present at wk 13 of animals in G3, G4, and G5.

Organ Weights: No treatment-related effects.

Bone marrow: No treatment-related effects.

Gross Path: No treatment-related effects.

Histopathology: No treatment-related effects.

Toxicokinetics: Plasma samples were collected on day 1 and during wk 13 (0.5, 1, 3, 5, 8, 12 and

24 hrs after dosing), and AUCs were determine for entacapone (E), the Z-isomer (Z), L-DOPA (LD), carbidopa (CD), and dopamine metabolites:

Cmax (μg/ml)

		20/20/5 (G2)	40/40/10 (G3)	80/80/20 (G4)	80/0/0 (G5)	0/80/20 (G6)
E	Day 1	1.9	2.7	2.8	3.5	---
	Wk 13	1.1	1.4	1.5	1.7	
Z	Day 1	0.5	0.6	0.7	0.8	
	Wk 13	0.3	0.3	0.3	0.5	
LD	Day 1	7.9	10.5	11.4		16.8
	Wk 13	7.9	10.5	16.6		34.5
CD	Day 1	0.3	0.3	0.4		0.8
	Wk 13	0.2	0.2	0.4		1.5

AUCs (0-inf, day 1; 0-24 hr wk 13)

		20/20/5	40/40/10	80/80/20	80/0/0	0/80/20
E	Day 1	2.0	4.4	5.0	6.7	
	Wk 13	1.4	2.3	3.4	3.4	
Z	Day 1	0.5	1.0	1.3	1.7	
	Wk 13	0.4	0.5	0.8	1.0	
LD	Day 1	12	19	30		37
	Wk 13	13	21	48		68
CD	Day 1	0.5	0.6	1.0		8.5
	Wk 13	0.8	1.9	4.4		19

Peak plasma levels of L-DOPA were lower in G4 versus G6 animals suggesting that E may delay or decrease L-DOPA absorption. Total LD exposure was also not increased by E, possibly because E inhibited the absorption of carbidopa, allowing more L-DOPA to be converted to DA; hence, the rise in DOPAC in G4 animals shown in the following table:

AUCs (0-inf, day 1; 0-24 hr wk 13)

		20/20/5	40/40/10	80/80/20	80/0/0	0/80/20
DOPAC	Day 1	8.7	16	23		17
	Wk 13	6.4	12	28		17
HVA	Day 1	5.4	9.7	14		17
	Wk 13	4.1	7.4	12		18
3-OMD	Day 1	7.2	13	24		110
	Wk 13	11	19	30		140

C.3.c. Combination Studies with L-DOPA and benserazide in Rats

Four- and 13-week studies were conducted, but a comprehensive review of these studies will not be presented since MADOPAR is not available in the U.S. However, one notable renal finding was observed (enlarged nuclei [minimal karyomegaly] in proximal tubular cells of the inner renal cortex) that deserves mention because of analogous findings in the longer-term rats studies, and in rat studies with the related compound tolcapone. The incidence is shown in the following table:

	4-week study		13-week study			
	600/50/50[a]		160/80/20[a]		160/0/0[a]	
	M	F	M	F	M	F
Enlarged nuclei in PCT	10/10	8/10	4/9	3/9	7/9	0/10

[a] Doses shown as entacapone/L-DOPA/benserazide mg/kg/day

Relative kidney weights tended to be increased in the groups that displayed the histopath changes.

The reason that this finding appeared in the study with benserazide and not with carbidopa can not simply be ascribed to dose or exposure based on toxicokinetic data. Plasma exposures to E and LD were generally similar with either decarboxylase inhibitor. In the benserazide study, plasma exposures to E were similar in males treated with only E and males treated with the combination. E levels in females treated with the combination appeared lower than those in females treated with E alone, which is also inconsistent with an exposure relationship. The observation that karyomegaly occurred at a higher incidence in females, and a lower incidence in males treated with the combination further complicates the interpretation of these findings.

The renal findings were not associated with major functional changes.

C.4. Chronic Toxicology

C.4.a. 52-Week Oral Toxicity of Entacapone (OR-611) in the Rat

GLP
Conducted by: Orion Research, Espoo, Finland
Research Report #: F91062870278
Sponsor Volumes: 1.16
Report Date: 8/24/93

Summary:

Entacapone (20, 90 and 400 mg/kg) was administered by gavage to Sprague-Dawley rats (n = 20) for 52 weeks. Mean terminal body weights were reduced by 10% in HDM. Some small (<10%), but significant changes in red cell parameters were evident, and are noted as consistent findings with other animal studies. Group mean changes in clinical chemistry parameters were noted (↑ alk phos, ↓Na, ↑ Pi), but not associated with histopathological findings. Urinary electrolyte alterations were of interest because of the subsequent finding of renal toxicity, but the changes were minor and transient. Relative adrenal, brain, heart, and liver weights were increased by 10%, and kidney weights were increased by 20% in HDM.

In the initial H-E tissue evaluation, minimal to slight, end-stage chronic fibrous myocarditis in males was reported as the only finding potentially related to treatment. This lesion was suggested as related to an exaggerated pharmacological effect, as endogenous catecholamines are thought to play a role in myocarditis that occurs with aging in rats (disruption of perfusion). The lesion could also have been secondary renal injury.

Apparently subsequent to the observation of renal tumors in the 2-year bioassay, a re-evaluation of kidney sections using a trichrome stain for hyaline droplets was undertaken to assess the involvement of altered renal handling of a male rat-specific protein (α2-microglobulin; α2-μG) in renal tumorigenesis. This method demonstrated a significant increase in staining intensity between control and HD males with the strongest staining in the P1-P2 region. No difference was evident between control and treated females. These findings provide some evidence that α2-μG deposition in renal tubular regions may be involved in the renal lesions that occur following entacapone. In addition, other renal lesions were identified in H&E-stained slides that were not reported as drug-related in the initial study (chronic progressive nephropathy, enlarged nuclei). [For further evaluation of the renal findings, see section C.7.c].

The NTE in this study is considered to be 90 mg/kg/day.

A limited analysis of plasma entacapone concentrations provided little information beyond demonstrating drug absorption.

Methods:

Animals:	Crl:CD Sprague-Dawley rats;	6 wks;	M: 198 ± 13g, F: 154 ± 10g
N:	20/sex/group (Lot G PUL 606 090)		
Dosages:	20, 90, 400 mg/kg/day		
Route/Veh:	Oral (gavage) in 0.5% methylcellulose (5 ml/kg)		
Lot:	Batch 005		

Results:

Mortality: 18 deaths occurred during the study. None were considered treatment-related.

Clin Obs: Yellow fur staining in MD and HD; urine staining at all doses (dose-related increase in intensity of staining).

Body Wt: Mean terminal body weights were reduced by 10% in HDM at wk 52 (not indicated as significant). No treatment-related effects occurred in females.

Food Intake: No consistent treatment-related effects.

Water Intake: Sig. increase over the last half of study in HDM.

Ophthalm (wk 25, 51): No treatment-related effects.

Hematol (wk 13, 27, 52): (n = 10/s/g)

Small (<10%), but significant changes in red cell parameters were evident, and are noted as consistent findings with other animal studies:

↓ Hb	-	HDM (wk 27, 52)
↓ Hct	-	HDM (wk 27, 52); MDF, HDF (wk 27)
↓ RBC	-	HDM (wk 27)
↑ MCHC	-	MDF, HDF (wk 27)

Platelets were decreased by 15% in HDF at wk 27.

Clin Chem (wk 13, 27, 52): (n = 10-11/s/g)

Potentially meaningful group mean changes included increased Alk Phos (HDF - wk 27, 52), decreased Na (HDF - wk 27, 52), and increased Pi (HDM and HDF - wk 27; HDM - wk 52). Some individual variations were sporadically seen, but were not identified as dose-related and did not correlate with significant histopathological findings.

Urinalysis (wk 13, 27, 52): (n = 10-11/s/g)

Potentially meaningful group mean changes were decreased Na (HDM, wk 13, 27), decreased Cl (HDM, wk 13, 27 (n.s.), decreased urine volume (MDF, HDF, wk 13).

Urinary erythrocytes and/or hemoglobin were seen in MD and HD animals using dipstick tests. The sponsor suggests that these findings may be artefacts due to the drug's intense coloration of the urine. Microscopy of urine samples failed to confirm the dipstick findings. Urine coloration interfered with analyses of bilirubin, nitrites, leucocytes and ketones.

Hepatic-NPSH: A slight increase of non-protein sulfhydryl groups was observed in HDF.

Organ Weights: Relative adrenal, brain, heart, and liver weights were increased by 10%, and kidney weights were increased by 20% in HDM. The changes were suggested as secondary to reduced body weight.

Gross Pathology: Yellow coloration of the fur and stomach occurred in MD and HD animals. Red spots were noted in the stomach of some HD animals.

Histopathology: Complete histopathology was conducted only on control and HD animals, premature MD decedents, and hearts of LD and MD animals.

In the initial H-E tissue evaluation, end-stage chronic fibrous myocarditis was reported as a possible treatment-related finding, based on a dose-related increase in frequency (4 ConM, 9 LDM, 9 MDM, 13 HDM). No females exhibited this lesion. The degree of severity was graded as minimal to slight.

In a follow-up study, kidney sections were re-evaluated using Martius Scarlet Blue, a trichrome stain for hyaline droplets. This method demonstrated a significant increase in staining intensity between control and HD males. The strongest staining was in the P1-P2 region. No difference was evident between control and treated females.

The follow-up study identified other changes in H&E-stained slides that were not reported as possibly drug-related in the initial study. It is noted that chronic nephropathy was seen in the initial evaluation in 2 ConM, 5 HDM, 2 ConF and 6 HDF; the discrepancy between the studies was not discussed by the sponsor.

	CON		ENTAC	
	M	F	M	F
Enlarged nuclei	1/20	8/20	18/20*	14/20
Chronic Prog. Nephropathy	3/20	0/20	11/20*	2/20

Plasma Concs (µg/ml): Samples were drawn 1 and 24 hr post-dose. In wk 51, most HD animals had detectable levels 24 hr post-dose (data not shown). The single time point provides little information beyond demonstrating proof of absorption.

	20		90		400	
	M	F	M	F	M	F
Wk 2	1.6	1.2	9.2	9.0	42.9	64.6
Wk 12	4.2	1.7	22.9	27.7	53.3	76.3
Wk 26	8.1	2.3	34.3	32.7	49.3	88.5
Wk 51	5.6	2.8	45.4	30.0	62.0	71.2

C.4.b. Entacapone (OR-611): Toxicity study by oral (capsule) administration to beagle dogs for 53 weeks

GLP
Conducted by : [redacted]

Research Report: 92/ORP015/0797
Sponsor Volumes: 1.18
Report Date: 6/22/93

Summary:

Entacapone (20, 80 and 300 mg/kg/day; final doses after 1-week escalation) was administered in a gelatin capsule to beagle dogs (n = 4/sex/dose) for 53 weeks. Body weight gain was reduced in MDF, HDM, and HDF, and food consumption was reduced at the HD. Consistent with studies in other species, mean red cell parameters (RBC, Hct, Hb, MCH, MCV) were reduced at the HD. This profile of hypochromic microcytic anemia is possibly related to an iron deficiency or iron chelation by entacapone. A few cases of altered red cell morphology (microcytosis, anisocytosis, hypochromasia), and a reduced number of erythroid cells in bone marrow smears were also observed. Statistically significant increases in relative kidney (HDF), salivary gland (HDM), and thyroid/parathyroid (HDM & F) weights were noted.

The sponsor did not consider any histopathology findings as treatment-related. However, 2 HDF showed evidence of renal tubular vacuolation and degeneration. This finding is noted for consideration of the sponsor's proposal that entacapone-induced renal lesions are a male rat-specific finding. Obviously, the occurrence of drug-related renal lesions in dogs is not consistent with this contention.

Toxicokinetic analyses revealed that entacapone does not accumulate in dogs, and that increases in exposure were greater than dose-proportional between 80 and 300 mg/kg, possibly because of saturation of first-pass metabolism. Conversion to the Z-isomer was 20%. Relative to plasma exposures in humans receiving the maximum recommended daily dose (200 mg, 8 times daily; AUC = 12 μg.hr/ml), entacapone exposures in dogs were:

LD: 0.3 times the human exposures
MD: 1.8 "
HD: 12.2 "

The NOAELs were 20 mg/kg/day in females and 80 mg/kg/day in males based on body weight reductions and/or erythron changes.

Methods:

Species: Beagle dogs; 16-20 wks old; 6.1 - 8.6 kg;
Number: 4/sex/group

Dosages: 0, 20, 80, 300 mg/kg/day were the final dosages.
Dosages were administered after a 1-week escalation phase. Day 1-3 doses were 25%, and day 4-7 doses were 50% of the final dose.

Route: Oral in gelatin capsules.
Lot: Batch 007[redacted]

Results:

Mortality: No animals died during the study.

Clin Signs: Dark feces, bright orange urine, and fur staining at MD and HD. Salivation in HDF. Active resistance to treatment in HD animals.

Body Wt/
Food Cons: Mean body weight gains were reduced in MDF (23%), HDF (36%) and HDM (18%). The effect in the HD group was largely attributable to two individual animals. Food consumption was also lower in the HD groups.

Ophthalm: (prestudy, wk 7, 13, 25, 39, 51)

No treatment-related effects.

EKG: (prestudy, wk 51; 1 and 24 hrs postdose)

No treatment-related effects.

Hematol: (prestudy, wk 7, 13, 25, 40, 51)

Significant group mean changes in red cell parameters were evident in HD animals over the course of the study. The specific changes were:

↓ Hb, Hct	(12-16%)	-	HDM (all time points)
	(9-15%)		HDF (wks 25, 40, 51)
↓ MCV	(6-12%)	-	HDM (wks 13, 25, 40, 51)
	(4-10%)		HDF (wks 25,40, 51)
↓ MCH	(9-13%)	-	HDM (wks 13, 25, 40)
	(9-13%)		HDF (wks 13, 25, 40, 51)
↓ RBC	(13%)	-	HDM (wks 7)

Platelet counts were significantly higher in HDF at all time points including prior to treatment.

Changes in red cell morphology were also observed in some HD animals:

microcytosis	-	1 HDM (4638), wk 13
		1 HDF (4601), wk 51
anisotosis	- -	1 HDM (4638), wk 25
		2 HDFs (4579, 4601), wk 25
hypochromasia	-	1 HDF (4601)

Bone marrow smears revealed a reduced number of cells of the erythroid series in 2 HDF (4579, 4601).

Clin Chem: (prestudy, wk 7, 13, 25, 40, 51)

No toxicologically significant findings

Urinalysis: (prestudy, wk 6, 12, 24, 38, 50)

Urine was stained from dark yellow to orange with the intensity dependent on dose and duration of treatment.

No other urine changes were identified by the sponsor as treatment-related, and the reliability of the tests is questionable because of the coloration artefact. However, animal 4579 had a protein level of 500 mg/dl. This animal also displayed hematological changes and renal tubular vacuolation and degeneration.

Organ Weights:

Statistically significant increases in group mean relative weights of kidney (44%) in HDF, submandibular salivary glands in HDM (28%), and thyroids/parathyroids in HDM (53%) and HDF (42%).

The increase in kidney weights was considered unrelated to treatment. Mean relative kidney weights were higher by 19% in HDM (not sig.) The group mean increases were attributable to one animal/sex (HDM #4664; HDF #4601), both of which had other abnormal, possibly drug-related findings that could be associated with the increase in renal weight:

4664 - chronic myocarditis
4601 - renal tubular vacuolation and degeneration, altered red cell morphology

In addition, the hematological profiles of these animals were consistent with iron-deficient anemia, except that reticulocytosis was not evident.

Gross Path: Yellow staining of the coat was evident in 1 MDF, 1 HDM, and all HDF. Dark GI contents were evident in 1 MDF, 1, HDM, and 2 HDF. One HDF was emaciated.

Histopath: The sponsor did not consider any histopathology findings "unequivocally related to treatment..." However, two findings that were observed only in HD animals should be noted as possibly drug-related based on the known toxicology of entacapone and related compounds:

renal tubular vacuolation and degeneration - 2 HDF (4579, 4601)

chronic myocarditis - 1 HDM (4664)

Since the renal finding occurred in 50% of the females, it is difficult to dismiss this finding

as unrelated to drug despite the lack of occurrence in any males. Both entacapone and tolcapone appear associated with renal pathologies in rats, although possibly by different mechanisms. As indicated above, two animals (including 4601) had abnormally high relative kidney weights. These findings raise further the possibility that renal injury in dogs could be related to drug.

Chronic myocarditis is noted as a finding consistent with the 1-year rat study.

Parafollicular hyperplasia of mandibular lymph nodes was identified in 1 HDM, 1 MDF, and 2 HDF. This finding may be associated with drug effects on salivation.

Toxicokinetic Analyses: (weeks 2, 26 and 50)

Exposures to entacapone did not increase over time, with the possible exception of HDF at wk 50. AUCs increased greater than dose-proportional between the MD and HD; the sponsor attributes this to possible saturation of first-pass metabolism. Conversion to the Z-isomer was approximately 20% based on AUC.

Cmax (µg/ml)

		20		80		300	
		M	F	M	F	M	F
Wk 2	E	1.1	2.3	3.2	6.4	23.1	12.1
Wk 26	E	1.7	2.1	8.0	5.0	14.3	22.2
Wk 50	E	2.2	2.1	2.7	3.8	19.7	18.9

$AUC_{(0\text{-}24)}$ (µg.hr/ml)

		20		80		300	
		M	F	M	F	M	F
Wk 2	E	2.2	3.6	15.6	28.7	141.0	86.7
	Z	0.3	0.6	3.7	6.0	39.2	25.5
Wk 26	E	2.9	4.2	30.6	25.0	124.0	96.2
	Z	0.5	0.8	6.8	4.3	31.5	29.7
Wk 50	E	3.8	4.2	20.3	22.1	142.0	153.0
	Z	0.5	0.5	5.6	4.3	35.1	41.5

C.5. Reproductive Toxicology

C.5.a. Entacapone: Study of fertility and early embryonic development to implantation in the rat

GLP Research Report: 94/0686 Sponsor Volume: 33
Conducted by: [redacted] Report Date: 2/24/95

Summary:

Entacapone was administered by gavage at doses of 20, 80 and 350 mg/kg, twice daily (40, 160, 700 mg/kg/day) to SD rats (22/sex/dose). Doses apparently were selected based on a range-study finding of reduced body weight gain at 350 mg/kg, b.i.d. Males were treated for 71 days prior to mating, and sacrificed after mating. Females were treated for 15 days prior to mating to 7 days post-coitum, and sacrificed on day 20 for delivery of pups by Caesarean section.

No treatment-related effects on body weight or food consumption were observed. No clinical signs were evident except for coloration of skin and urine, and salivation at the HD. Fertility was not impaired by entacapone, but 4 HDF required two estrus cycles to mate. There were no drug-related changes in gross pathology of F_0 or in litter parameters. The sponsor did not consider any changes in fetal morphology as treatment-related, but some anomalies, mainly affecting the eyes, were noted as occurring at a higher incidence in treated versus control animals, and higher than historical control ranges.

Toxicokinetics were not determined in this study, but in a preliminary Seg II study, estimated maternal plasma exposures at a dosage of 350 mg/kg, b.i.d. on day 15 of gestation ($AUC_{0\text{-}10\ hr}$ multiplied by 2 = 450 µg.hr/ml) exceeded estimated human exposures at the maximum recommended daily dose (200 mg, 8 times daily; AUC = 12 µg.hr/ml) by 37 times.

An MTD was not acheived in this study, although the previous range-finding had demonstrated an effect on body weight at the high dose. In the absence of clear signs of toxicity, the validity of the study from a toxicological standpoint is questionable.

Methods:

Species: Crl:CD rats; M: 210 - 248g, 6-7 wks; F: 214 - 260g, 10-11 wks;
Number: 22/sex/dose
Dosages: 0, 20, 80, 350 mg/kg, b.i.d. (40, 160, 700 mg/kg/day)

Doses apparently were selected based on a range-finding Seg I/III study of 200, 350, and 500 mg/kg, b.i.d. The endpoint from that study was not clearly stated by the sponsor, but body weight gains were reduced by 16 and 20% in MDM and HDM during the treatment period (15 days premating to lactation day 4). Body weight gains in females were not reduced during the premating period, but were reduced by 8% and 23% in MDF and HDF during gestation. No other major adverse effects were observed in that study except for minor reductions in RBCs and Hb.

Route/Veh: Oral gavage in 0.5% methylcellulose (10 ml/kg)
Lot: Batch 011[redacted]

Treatment Schedule:

Males: For 71 days prior to mating, throughout the mating period until termination after necrospy of females.

Females: From 15 days prior to mating, throughout mating until day 7 post-coitum. Females were sacrificed on day 20 of gestation for examination of uterine contents.

Reproductive/Developmental Assessments:

Males: Gross pathology. Sperm sample withdrawn for motility and morphology analysis. Sex organs preserved and examined (Con and HD).

Females: Number of corpora lutea, number of implantations, resorptions (early, late), and fetuses (distribution in horn).

Fetuses: Weight, sex, external abnormalities (all), skeletal (1/2 of subjects; Alizarin red staining) and visceral (1/2 of subjects; Wilson's method) abnormalities.

Results:

F_0 Generation

Mortality: None

Clin Obs: Yellow staining of body and salivation in HDM and HDF. Colored urine in all treated animals.

Body Wt: No treatment-related effects on body weight gain in males or females.

Food/Water Intake: No treatment-related effect on food intake; increased water consumption at HD.

Reproductive Performance:

Conception rates and fertility indices were 100%. Most matings occurred during the first estrus cycle, except for 4 HDF that mated in the second cycle, and 1 MDF that was acyclic (precoital interval of 17 days).

Male Autopsy:

Gross path findings were limited to yellow coloration of body, and bladder contents, and dark cecal contents at MD and HD. There were no effects on organ wts, sperm count, motility or morphology, or histopathology of reproductive organs.

Dam Autopsy:

Gross path findings in dams were similar to those seen in males.

There were no treatment-related effects on number of corpora lutea, implantations, resorptions, pre- or post-implant loss, viable young, or fetal and placental weights.

Fetal Examinations:

The sponsor concluded that there were no treatment-related effects on fetal morphology. The following anomalies, mainly affecting the eyes, were noted as occurring at a higher incidence in treated versus control animals, and higher than historical control ranges (* except where marked). The findings were considered "fortuitous", since the teratology study of higher doses revealed no treatment-related increases in the incidence of visceral anomalies.

% Fetal Incidence (# litters affected; 22/group)

		0	40	160	700	historical
External		n = 342	n =356	n = 356	n = 350	
	squat fetus; bil. forelimb flexure	0	0	1.4 (2)	1.1 (1)	#
	cleft palate	0	0	0	0.3 (1)	0.0 - 0.3
	2 fetuses/1 placenta	0	0	0	0.3 (1)	#
Visceral		n = 166	n = 172	n = 174	n = 170	
	unil. sl. macrophthalmia	1.2 (2)	1.2 (2)	2.3 (4)	2.9 (4)	0.0 - 1.9
	unil. anophthalmia	0	0	0	0.6 (1)*	0.0 - 1.4
	bil. sl. macrophthalmia	0	0	0.6 (1)	0	#
	bil. sl. microphthalmia	0	0	0	1.2 (1)	#
	internal hydrocephaly	0	0	0.6 (1)	1.8 (1)	0.0 - 0.6

no previous background data; Doses expressed as mg/kg/day

There were no treatment-related skeletal anomalies.

C.5.b. Entacapone: Study for effects on embryo-foetal development in the rat

GLP Research Report #: 94/0583 Sponsor Volume: 34

Conducted by: [redacted] Report Date: 3/15/95

Summary:

Entacapone was administered to pregnant female rats (N = 22) by gavage at doses of 20, 80, and 500 mg/kg, b.i.d. (40, 160, 1000 mg/kg/day) during the period of organogenesis (days 6-15 of gestation). Doses were selected based on a range-finding Seg II study of up to 1000 mg/kg/day; no toxicities were observed in that study, but drug absorption appeared to approach saturation at 350-500 mg/kg.

No effects of entacapone on body weight, food intake, clinical signs of toxicity, litter parameters or gross pathology of dams were evident. There were no apparent effects on external or visceral fetal examinations. Some delays in ossification were noted, as were increased incidences of enlarged fronto-nasal suture and wavy ribs at the highest test dose. These potential treatment-related findings are noted to occur at doses below maternotoxic levels.

The NOAEL for F_1 was 160 mg/kg, and the NOAEL for F_0 was 1000 mg/kg/day. TK analyses indicated that maternal exposures on day 15 exceeded estimated human exposures at the maximum recommended dose (200 mg/kg, 8 times daily) by approximately 5- and 68-fold, respectively.

Methods:

Species: Crl:CD rats; 220 - 268g; 10-11 wks;

Number: 22 pregnant rats/dose + 4/dose for TK analysis

Dosages: 0, 20, 80, 500 mg/kg, b.i.d. (40, 160, 1000 mg/kg/day)

Doses apparently were selected based on a range-finding Seg II study of 200, 350, and 500 mg/kg, b.i.d. (400, 700, 1000 mg/kg/day). No clearly treatment-related effects were observed in that study, except for yellow coloration of body surface and cage trays (i.e., an MTD was not acheived). However, a TK analysis suggested that absorption may approach saturation at the higher dose levels (following Figure and Table from Sponsor).

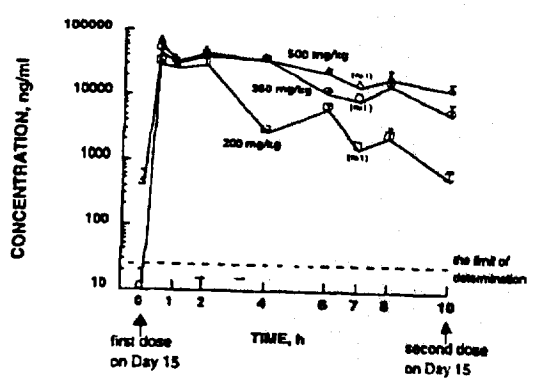


Figure 28. Concentrations of entacapone in plasma of pregnant rats after the first daily dose on Day 15 of gestation following repeated oral dosing of entacapone twice daily for 10 days (Mean, SEM, n=4/time point).

The peak plasma concentrations, the AUC values for the first dosing interval of entacapone in rats on Day 15 of gestation and the exposure factors achieved following repeated oral dosing of entacapone for 10 days.

Dose		Parameter after the first daily dose on Day 15		Exposure factor 1)
twice daily mg/kg	total mg/kg/day	Peak conc. i.e. $C_{30\ min}$ (µg/ml)	AUC^{0-10h} (h·µg/ml)	
200	400	29.2	94	21
350	700	46.3	224	50
500	1000	61.2	275	61

Calculated from the mean entacapone concentration - time data (4 rats/time point).
1) Exposure factor was calculated by dividing AUC_{rat} by AUC_{man}. The AUC values were derived by multiplying the AUC of entacapone after a single dose (in man 1.5 h·µg/ml) with the number of the dosing frequency, i.e. in the rat with 2 and in man with the average which is 6.

Route/Veh: Oral gavage in 0.5% methylcellulose (10 ml/kg)
Lot: Batch 011[redacted]

Regimen: Animals were dosed twice daily (10 hrs apart) on days 6-15 of gestation. Dams were sacrificed on gestation day 20.

Assessments:

Dams: Number of corpora lutea, implantations, and resorptions (early, late); number and distribution of fetuses in uterine horn.

Fetuses: weight, sex, external abnormalities (all), skeletal (1/2 of subjects; Alizarin red staining) and visceral (1/2 of subjects; Wilson's method) abnormalities.

Results:

F_0 Generation

Mortality: None

Clin Obs: Yellow staining of body and salivation at HD. Colored urine in all treated animals.

Body Wt: HDF lost weight after the first treatment day, but subsequently recovered. Overall weight gain during gestation was unaffected by treatment.

Food/Water Cons: Food intake was unaffected by treatment. Water intake was increased in HD.

Gross Path: Aside from yellow staining of body at MD and HD, there were no treatment-related effects.

Litter Params: All animals were pregnant. There were no treatment-related effects on number of corpora lutea, implantations, viable young or resorptions. Placental weights were slightly higher at the HD (8%), but within historical control.

Fetal Exams: There were no indications of potential treatment-related external or visceral abnormalities. Some skeletal findings, mostly ossification delays, tended to occur at higher than control incidence (within test and/or historical) in HD groups. The sponsor considers the relationship of these findings to treament "unclear". They are noted to occur at doses lower than maternotoxic levels.

% Fetal Incidence (# litters affected; 22/group)

	0	40	160	1000	historical
	n = 178	n = 183	n = 180	n = 173	
incomplete oss., parietal bone	1.7 (2)	0	1.7 (2)	7.5 (5)	0.0 - 5.7
" , squamosal bone	3.4 (4)	1.1 (2)	2.2 (4)	8.7 (5)	0.0 - 4.1
" , frontal bone	0.6 (1)	0	0	2.9 (3)	0.0 - 1.8
" , jugal bone	0	0	0	2.9 (4)	0.0 - 2.8
fronto-nasal suture enlarged	1.7 (3)	1.6 (3)	2.2 (3)	6.4 (5)	0.0 - 3.0
wavy ribs	0	0	0.6 (1)	1.7 (2)	0.0 - 0.7
incomplete oss., cerv. vert. arches	0	0	0	2.3 (3)	0.0 - 0.8
" , sacral vert. arches	0	0	0	3.5 (2)	0.0 - 4.3
" , ischial bones	0.6	0.5	0.6	2.9 (2)	0.0 - 2.8

Doses expressed in mg/kg/day

Toxicokinetics: Plasma samples were collected from satellite animals (n = 4) at 3-4 time points after dosing on days 6 and 15. Therefore, the TK values presented are rough estimates:

		40	160	1000
$C_{30\ min}$ ($\mu g/ml$)	day 6	4	28	48
	day 15	5	32	69
AUC* ($\mu g.hr/ml$)	day 6	6	55	360
	day 15	6	60	410

* 0-inf on day 6 for LD & MD; 0-10 hr on day 6 for HD, and for all groups on day 15

C.5.c. Entacapone: Study for effects on embryo-foetal development in the rabbit

GLP Research Report #: 94/0561 Sponsor Volume: 35

Conducted by: [redacted] Report Date: 3/15/95

Summary:

Entacapone was administered by gavage at doses of 20, 50 and 150 mg/kg, b.i.d. (40, 100, 300 mg/kg/day) to pregnant New Zealand white rabbits (22/dose) during organogenesis (day 6 to 19 of gestation). Does were sacrificed on day 29 for delivery of fetuses by Caesarean section. Doses were based on a preliminary teratology study in which lethality occurred at 700 mg/kg.

The main drug-related finding was induction of abortions in 1 MD and 2 HD does. HD animals lost weight during gestation. Body weight gain in MD animals was suppressed during gestation, but recovered after treatment cessation. There were no statistically significant changes in reproductive or litter parameters, but the incidence of late and total resorptions, and the percentage of post-implantation losses appeared higher in MD and HD does than in control and LD does, and outside of the historical ranges. A dose-related reduction in fetal and placental weights was not statistically significant, but corresponded to a higher incidence of small fetuses at the HD. The only other fetal finding was ossification delays at the HD.

The NOAEL was 40 mg/kg/day for F_0 (abortions, reduced body weight) and for F_1 (post-implantation losses). Toxicokinetic analyses indicated that estimated maternal plasma entacapone exposures at the HD exceeded estimated human exposures at the maximum recommended daily dose (200 mg, 8 times daily) by 1.6 times. Maternal exposures at the NOAEL were well below expected human exposures.

Methods:

Species: New Zealand white rabbits; 2.54 - 3.64 kg; 21 - 29 wks;

Number: 22 pregnant rats/dose + 4/dose for TK analysis

Dosages: 0, 20, 50, 150 mg/kg, b.i.d. (40, 100, 300 mg/kg/day)

Dosages were based on a range-finding teratology study of 400, 700 and 1000 mg/kg/day on day 6-19 of gestation. Three of four MD, and all HD animals died in that study; the surviving MD was not pregnant. The cause of death was not identified. Food and water consumption, but not body weight gain, was reduced during treatment at the LD. Fetal and placental weights were reduced in the offspring of LD dams.

Route/Veh: Oral gavage in 0.5% methylcellulose (5 ml/kg)

Lot: Batch 011[redacted]

Regimen: Animals were dosed twice daily (10 hrs apart) on days 6-19 of gestation. Dams were sacrificed on gestation day 29.

Assessments:

Dams: Number of corpora lutea, implantations, and resorptions (early, late); number and distribution of fetuses in uterine horn.

Fetuses: Weight, sex, external abnormalities (all), skeletal (all completely examined by Alizarin red staining; 1/3 of heads examined after free-hand serial section) and visceral exam (gross examination of neck, thoracic and abdominal cavities).

Results:

F_0 Generation

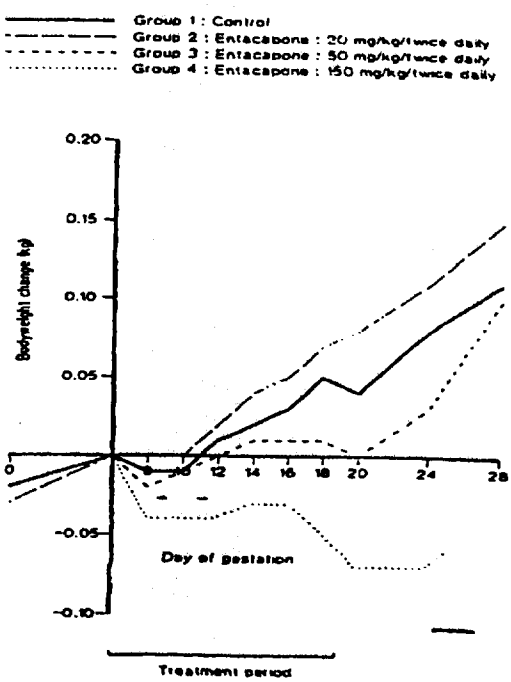
Mortality: One HD dam was found dead on GD23. A lobular pattern of the liver, and a GI disturbance was noted. The death was considered unrelated to treatment.

One Con dam was sacrificed moribund after suffering a maladministration.

Abortions: One MD and 2 HD aborted. No treatment-related findings were evident at necropsy.

Clin Obs: Yellow staining of limbs and reduced fecal output at HD. Colored urine in all treated animals.

Body Wt: HD animals tended to lose weight during the treatment period. Weight in MD does also appeared lower than controls or remained constant after the first treatment day, but subsequently recovered.



Food/Water

Cons: Food and water intake was reduced in HD animals during treatment, but recovered upon dosing cessation.

Gross Path: There were no treatment-related findings, aside from yellow staining of body surfaces.

Litter Parameters:

The number of pregnant females, corpora lutea, and implantation sites were comparable in all groups. The incidence of late and total resorptions, and the percentage of postimplantation losses appeared higher in MD and HD does than in control and LD does, and outside of the historical ranges. However, the sponsor did not consider the changes biologically relevant, since the differences were not statistically significant (sponsor Table 7A).

TABLE 7B

Uterine examination - group mean foetal and placental weights

Group		Foetal weight (g)			Placental weight (g)
		Male	Female	Overall	Overall
1	Mean	41.2	40.2	41.0	5.4
	SD	2.2	2.3	1.7	0.3
	n	20[a]	21	21	21
2	Mean	41.2	40.6	40.7	5.3
	SD	2.3	2.7	2.0	0.3
	n	19	19	19	19
3	Mean	39.1	38.1	39.0	4.7
	SD	3.2	3.0	2.1	0.4
	n	18	17[b]	18	18
4	Mean	37.1	36.3	37.0	4.9
	SD	3.2	2.1	2.2	0.4
	n	18	18	18	18
Background control (4 studies)					
Mean		41.5	41.1	41.2	5.0
Low		37.6	37.8	37.6	4.1
High		45.0	44.5	44.6	5.8

SD Standard deviation.
n Number of litters.
a One litter with no males present.
b One litter with no females present.

A reduction in fetal and placental weights appeared dose-related, but did not achieve significance at the MD or HD (sponsor Table 7B).

TABLE 7A

Uterine examination - group mean values for females killed on Day 29 of gestation

Group	Number of pregnant animals		% Abortion and total litter loss	Corpora lutea count	Implantations	Viable young			Resorptions			Implantation loss (%)	
						M	F	Total	Early	Late	Total	Pre-	Post-
1	21	Mean	0.0	10.0	8.8	3.9	4.2	8.0	0.5	0.2	0.7	13.2	8.2
		SD		2.7	3.0	1.7	1.9	2.9	0.7	0.5	0.8		
2	19	Mean	0.0	10.2	8.8	4.5	4.0	8.5	0.1	0.2	0.3	13.9	3.6
		SD		2.3	2.3	2.0	1.6	2.1	0.3	0.5	0.6		
3	19	Mean	5.3	11.2	10.2	4.8	3.9	8.7	0.5	0.9	1.4	12.0	14.2
		SD		2.9	3.0	1.7	2.2	2.7	0.7	1.0	1.2		
4	21	Mean	9.5	9.5	8.4	2.9	4.2	7.1	0.6	0.7	1.3	11.7	15.2
		SD		1.7	1.9	1.4	1.6	1.8	0.7	0.8	1.1		
Background control (4 studies)													
Mean				9.3	8.0	3.6	4.0	7.6	0.33	0.20	0.50	13.9	6.3
Low				8.7	7.5	2.9	3.3	6.5	0.00	0.00	0.00	4.4	0.0
High				9.9	8.6	4.7	4.7	8.0	0.80	0.40	1.00	24.0	13.3

SD Standard deviation.

Fetal Evaluation:

Potential treatment-related findings from the external, visceral, and skeletal examination are shown in the following table. The higher incidence of small fetuses is consistent with the reduced fetal weights shown above. The findings of incomplete ossification may represent a developmental delay secondary to reduced weight development.

% Fetal Incidence (# litters affected)

	0	40	100	300	historical
number of fetuses examined " " litters "	169 21	161 19	157 18	128 18	
small fetus (< 32 g)	19.5 (7)	8.7 (7)	17.8 (12)	30.5 (12)	1.2 - 17.4
incomplete oss., heads of limb long bones " , metacarpals or phalanges " /short pollices	52.1 (18) 9.5 (6) 1.2 (2)	55.9 (18) 6.8 (4) 3.1 (3)	54. 1 (16) 3.2 (2) 1.3 (2)	65.6 (18) 14.1 (7) 7.0 (4)	35.6 - 52.2 3.5 - 5.8 0.0 - 2.9

Doses expressed in mg/kg/day

Toxicokinetics:

Plasma samples were collected from satellite animals (n = 4) at 5-6 time points after dosing on days 6 and 19.

		40	100	300
$C_{15\ min}$ (µg/ml)	day 6	1.3	5.6	19.5
	day 19	1.8	4.2	4.3
AUC* (µg.hr/ml)	day 6	1.0	3.8	22.9
	day 19	1.4	3.2	9.3

* 0-inf on day 6; 0-10hr on day 19

Concentrations of the Z-isomer were not determined, but peaks were detected on the chromatograms. The sponsor considers conversion to the Z-isomer to be very low in rabbits.

C.5.d. Entacapone: Study for effects on pre- and post-natal development in the rat

GLP Research Report: 94/ORP043/0813 Sponsor Volume: 35

Conducted by: [redacted] Report date: 4/5/95

Summary:

Entacapone was administered by gavage at doses of 20, 80, 350 mg/kg, b.i.d. (40, 160, 700 mg/kg/day) to pregnant female rats (n=22) from day 7 of gestation to lactation day 20. Dams were sacrificed at weaning. Culled pups were reared to maturity to assess maturational development, including neurobehavioral and reproductive function. Doses were selected based on a range-finding study in which body weight gains were reduced by 8% and 23% in females treated with 350 or 500 mg/kg during gestation.

Entacapone did not produce any signs of maternotoxicity (decreased body weight or food consumption), and there were no significant effects of entacapone on pre- and post-natal development, or growth and maturation of the offspring.

Toxicokinetics were not determined in this study. Based on the toxicokinetic analysis in a preliminary Seg II study (see p. 40), estimated maternal plasma exposures at a dosage of 350 mg/kg, b.i.d. on day 15 of gestation ($AUC_{0\text{-}10\ hr}$ multiplied by 2 = 450 µg.hr/ml) exceeded estimated human exposures at the maximum recommended daily dose (200 mg, 8 times daily; AUC = 15 µg.hr/ml) by 37 times.

In the absence of overt signs of maternotoxicity, the validity of the study is questionable. Toxicokinetic data suggesting that drug absorption approaches saturation in the range of 350-500 mg/kg may be acceptable to support HD selection, but total drug-related materials were not determined.

Methods:

Species: Crl:CD rats; 233 - 285g; 10-11 wks;

Number: 22 pregnant rats/dose + 4/dose for TK analysis

Dosages: 0, 20, 80, 350 mg/kg, b.i.d. (40, 160, 700 mg/kg/day)

Doses apparently were selected based on a range-finding Seg I/III study of 200, 350, and 500 mg/kg, b.i.d., in which body weight gains were reduced by 16 and 20% in MDM and HDM during the treatment period (15 days premating to lactation day 4). Body weight gains in females were not reduced during the premating period, but were reduced by 8% and 23% in MDF and HDF during gestation.

Route/Veh: Oral gavage in 0.5% methylcellulose (10 ml/kg)

Lot: Batch 011 [redacted]

Regimen: Animals were dosed twice daily (10 hrs apart) from gestation day 7 to lactation day 20. Dams were sacrificed after weaning or total litter death.

Assessments:

Dams: Clinical signs, body wt, food and water cons, gross path observations at sacrifice, number of implantation sites.

Litter parameters: Body wts, live/still births, and gross abnormalities were recorded day 1. Litters were culled to eight (4M, 4F) on day 4.

Developmental:

Maturational development was recorded (pinna unfolding, hair growth, incisor eruption, eye opening, vaginal and preputial separation). Functional responses (auditory startle, pupil contraction) were evaluated on LD 25. Locomotor activity, learning (water-filled Y-maze), and neuromuscular assessments were conducted between days 26-30.

Reproductive assessments were conducted at approximately 5 weeks of age in 20 animals/sex/group.

Results:

Effects on Dams

Mortality: One control female.

Clin Obs: Aside from yellow staining of the body, there were no treatment-related effects.

Body Wt: There were no treatment-related effects on body weight gain during gestation or lactation.

Food and Water Cons: There were no treatment-related effects on food consumption during gestation or lactation. Water consumption was increased in HD animals throughout gestation, and occasionally during lactation.

Gestation: There were no treatment-related effects on gestation length or parturition.

Necropsy: Yellow staining of body surfaces at the MD and HD were the only treatment-related gross path observation.

Effects on F_1

Litter Parameters

There were no treatment-related effects on litter size and survival, sex ratio or number of implantation suites. Body weight gains of HD pups were slightly reduced (6%) over days 1-28 of lactation.

Developmental assessments:

There were no treatment-related effects on maturational or functional indices, including neuromuscular, neurobehavioral, and reproductive assessments of the F_1.

Necropsy:

There were no treatment-related adverse gross pathology findings in F_1 generation

C.6. **Genetic Toxicology**

a. Entacapone: *Salmonella typhimurium* and *Escherichia Coli* Reverse Mutation Assay

b.1. Entacapone: Mouse Lymphoma TK Locus Assay

b.2. Entacapone:Mutation at the Thymidine Kinase (tk) Locus of Mouse Lymphoma L5178Y Cells using the Microtitre Fluctuation Technique

c. *In vitro* assessment of the clastogenic activity of OR-611 in cultured human lymphocytes

d. OR-611: Assessment of clastogenic action on bone marrow erythrocytes in the micronucleus test

e. Entacapone: In vivo rat liver DNA repair test

f. Binding of Radioactivity from ^{14}C-Entacapone to DNA

g. Entacapone: Reverse mutation in four histidine-requiring strains of *Salmonella typhimurium* and two tryptophan-requiring strains of *Escherichia Coli* in the presence of carbidopa and L-dopa

h. Entacapone: induction of micronuclei in the bone marrow of treated mice in the presence of carbidopa and L-dopa

C.6.a. Entacapone: *Salmonella typhimurium* and *Escherichia Coli* Reverse Mutation Assay

GLP; Report #: F96091210685 Report Date: 10/8/96 Volume: 36

Summary:

Entacapone was not mutagenic with or without metabolic activation in a battery of *Salmonella typhimurium* strains that was appropriate for detecting different types of mutations. Both the standard Ames test and the liquid preincubation modification was used. The highest test concentrations for the direct plate incorporation test were appropriate based on evidence of cytotoxicity; cytotoxicity was not achieved under all conditions by the preincubation method. Positive controls produced the expected results.

ethods:

ıg concentrations: (Batch 006; prepared in DMSO)

Test 1- direct plate incorporation: 31.25, 62.5, 125, 250, 500, 1000, 1500, 2000 µg/plate

Test 2- preincubation: 15.625, 31.25, 62.5, 125, 250, 500, 1000, 1500, 2000 µg/plate

Preliminary cytotoxicity testing (thinning of background lawn) was conducted to determine the top concentrations for each strain under each condition. For the direct plate method, cytotoxicity generally occurred at 1500-2500 µg/plate. However, cytotoxicity was not observed at the highest test concentrations under several test conditions using the preincubation method:

- TA98 and TA100 in the absence or presence of S9
- TA1535, TA1537, WP2 pKM101 and WP2 *uvrA* pKM101 in the absence of S9

Strains/Positive controls/Vehicles:

Strain	Sensitivity	Positive Control	Conc (DMSO)
TA 1535	base-pair substitution	sodium azide	2 µg/plate
TA 1537	frameshift mutation	9-aminoacridine	25-50 µg/plate
TA 100	base-pair substitution	methylmethane sulfonate	2 µl/plate
TA 98	frameshift mutation	2-nitrofluorene	5-10 µg/plate
WP2 pKM101	base-pair substitution	4-nitroquinoline-1-oxide	2 µg/plate
WP2 *uvrA*pKM101	base-pair substitution	"	1 µg/plate

Metabolizing System: S9 fraction prepared from Arochlor 1254-induced rats

Results:

No signs of increased mutation frequency were evident under any of the test conditions with entacapone.

Positive controls produced the expected increases in revertant frequency.

Conclusion:

Under the test conditions, entacapone was not mutagenic. It is noted that certain strains were not tested up to cytotoxic concentrations using the preincubation method. Since this was a secondary assay conducted in an attempt to enhance sensitivity, this deficiency is not considered to affect the overall conclusion.

C.6.b.1. **Entacapone: Mouse Lymphoma TK Locus Assay**
GLP; Report #: 39/930295 Report date: 12/17/93 Vol: 36
Conducted by: [redacted]

C.6.b.2. **Entacapone: Mutation at the Thymidine Kinase (tk) Locus of Mouse Lymphoma L5178Y Cells using the Microtitre Fluctuation Technique**
GLP; Report #: 544/37-1052 Report date: 11/95 Vol: 36
Conducted by: [redacted]

Summary:

The mutagenic/clastogenic effects of entacapone were tested in a mammalian cell culture system using two different methods (agar and microtitre fluctuation techniques). Drug test concentrations were appropriately selected to achieve cytotoxicity. Both methods yielded positive results, although the profiles differed slightly. The overall conclusion was that entacapone was positive in the ML/TK assay in the absence and presence of S9. Colony sizing showed that the major effect of entacapone was on small colony formation indicating chromosomal damage.

Methods:

Batch: 009[redacted] in DMSO (final DMSO conc = 2%).

Drug Concentrations:
Toxicity testing: 25 - 3000 µg/ml
Mutagenicity testing: 10 - 400 µg/ml (-S9)
1 - 100 µg/ml (+S9)

Positive Controls:
without activation - 4-nitroquinoline-1-oxide
with activation - benzo(a)pyrene

Metabolic Activation: S9 fraction from Arochlor-induced rats.

Experimental Procedure:

Cells were incubated for 3 hrs with entacapone with or without S9, washed and cultured for a 2 day expression period. Cells were then assessed for viability and mutant frequency by plating in agar or medium (Microtitre fluctuation technique to assess large and small colony formation). In the main experiments, cultures were run in duplicate. Single cultures were used in the preliminary experiments. The incubation period was approximately 12 days until scorable for large and small colonies.

Results

Combined results from the two experiments under the various test conditions is provided in the following Table. The level of cytotoxicity achieved at the highest test concentrations was in the acceptable range (10-20%), except for one assay with the microtitre method (40% RS at 150 µg/ml). The two methods (agar and microtitre) gave slightly different results. While both

demonstrated that entacapone caused significant increases in mutation frequency, a clear dose-relationship was not evident with the agar technique. Also with the agar technique, the effect of entacapone was more evident in the absence of S9, whereas similar increases in mutant frequency were observed in the absence and presence of S9 using the microtitre technique.

The microtitre assay revealed that a greater proportion of small colonies were formed.

Technique	Assay #	+/- S9	[E] (µg/ml)	% RS	↑MF	sig.
Agar	1	-	25	101	2.1x	*
			50	52	1.8x	*
			100	38	1.5x	*
			200	19	2.3x	*
			400	10	2.9x	*
		+	2.5	73	1.2x	*
			25	57	1.5x	*
			75	21	1.4x	*
			100	19	1.9x	*
	2	-	10	114	-	
			25	53	2.6x	*
			75	19	2.1x	*
			100	14	2.6x	*
		+	10	102	-	
			25	51	1.8x	*
			50	14	1.8x	*
			100	11	2.0x	*
Microtitre	1	-	6.25	83	1.4x	
			12.5	83	1.2x	
			25	70	2.4x	*
			50	40	2.5x	*
			100	18	3.9x	*
			200	6		
		+	6.25	87	-	
			12.5	93	1.1x	
			25	96	1.4x	
			50	26	2.9x	*
			100	10	3.5x	*
			200	2		
	2	-	12.5	96	1.1x	
			25	78	1.1x	
			50	59	1.8x	*
			100	35	1.6x	*
			150	40	2.2x	*
		+	12.5	89	-	
			25	88	1.2x	
			50	37	1.5x	*
			100	12	1.8x	*
			150	15	2.2x	*

C.6.c. In vitro assessment of the clastogenic activity of OR-611 in cultured human lymphocytes
GLP; Report #: B-154,840; Report date: 11/9/90 Vol: 36
Conducted by: [redacted]

Summary:

Entacapone was tested for clastogenic effects in cultured human lymphocytes at concentrations that produced less than a 50% reduction in mitotic index. Entacapone was clearly clastogenic in the presence of S9. Chromatid breaks were the most common aberration.

Methods:

Drug Concentrations and Exposures: Batch 003 [redacted] in DMSO

Prelim Tox Tests:	8-5000 and 8-800 µg/ml, +/- S9	
Main Test:	-S9: 0, 5, 10, 20 µg/ml;	24 hr exposure
	+S9: 0, 100, 200, 400 µg/ml;	3 hr exposure, harvest at 24 hr

Positive Controls/vehicles:

Without S9 activation:	Chlorambucil
With S9 activation:	Cyclophosphamide

Metabolizing System: S9 fraction prepared from Arochlor-induced rats (500 mg/kg).

Scoring: Chrome abs scored from 100 metaphases

Statistics: Fisher's Exact test ($p<0.05$; one-tailed test)

Results:

Mitotic index: The reductions in mitotic index were less than 50% under all test conditions

-S9: 41% at 20 µg/ml,
+S9: 28, 22, and 10% at 100, 200 and 400 µg/ml (inverse dose-relationship)

Clastogenicity: +S9: sig. ↑ in aberrant metaphases at 400 µg/ml (17% including and excluding gaps); chromatid breaks were most common finding.

-S9: no sig. ↑ in chrome abs.

Conclusion:

Entacapone was clastogenic in the presence of metabolic activation under the conditions of the assay. These effects were seen at drug concentrations that were not excessively cytotoxic.

C.6.d. OR-611: Assessment of clastogenic action on bone marrow erythrocytes in the micronucleus test

GLP; Report #: 91/ORP008/0116 Report date: 2/4/91 Vol: 36
Conducted by:

Summary:

Entacapone was administered by gavage at doses of 40, 200, 1000 mg/kg, or 35 mg/kg intravenously, to CD-1 mice for assessment of micronuclei formation in bone marrow erythrocytes. The oral high dose was selected based on the occurrence of lethality at 2000 mg/kg; doses between 1000 and 2000 mg/kg were not investigated. Animals were sacrificed 24, 48, and 72 hrs after treatment for scoring of erythrocytes.

Entacapone did not induce micronuclei formation under any test condition. Bone marrow toxicity was not evident; thus, the validity of the test model was not established.

Methods:

Doses: Entacapone (Batch 003) was administered by gavage at doses of 40, 200 or 1000 mg/kg (1.2% methylcellulose), or intravenously at a dose of 35 mg/kg (PBS). Chlorambucil (30 mg/kg) was the positive control.

The high oral dose was selected on the basis of a preliminary toxicity study in which lethality occurred in 1 of 2 females treated with 2000 mg/kg.

Animals: CD-1 mice; 18-26 g; 4-5 wks old;
N: 5/sex in LD, MD; 15/sex in Control and HD

Animals treated with the LD and MD were sacrificed 24 hrs post-treatment. Control and HD animals were sacrificed at 24, 48 and 72 hr post-treatment

Sample Collection and Analysis:

Femoral bone marrow samples were collected and smeared, and 1000 mature and 1000 immature erythrocytes were examined for the presence of micronuclei (Note: Deviation from OECD guidance that 2000 immature erythrocytes should be scored).

Results:

No entacapone treatment groups had frequencies of micronuclei that were greater than controls. Bone marrow toxicity was not observed. The positive control produced the expected result.

Conclusion:

Entacapone did not induce micronuclei formation in mice under the conditions of the study. Because of the absence of bone marrow toxicity, the test may not be appropriate.

C.6.e. Entacapone: In vivo rat liver DNA repair test

GLP; Report #[redacted]58/941422; Report date: 6/10/94; Vol: 36

Conducted by: [redacted]

Summary:

Entacapone (Batch 009) was tested for induction of DNA repair in hepatocytes from rats (Harlan Hsd/Ola (SPF); 140-149g; 5 wks old; N = 8M/dose) treated with a single oral gavage dose of 600 or 2000 mg/kg (1% methylcellulose) in the main study. The HD is the maximum recommended dose according to OECD guideline. A preliminary study demonstrated that this dose was tolerated.

Animals were sacrificed 2 or 14 hrs after treatment (n = 4/time point). Hepatocytes were isolated and cultured with [^{3}H]-thymidine for 4 hrs, chased with unlabeled thymidine for 24 hrs, washed, fixed, stained, and quantified by autoradiography.

Entacapone did not increase gross or net nuclear grain counts at any dose or expression time.

Conclusion:

Entacapone did not produce evidence of DNA damage under the conditions of this study.

C.6.f. Binding of Radioactivity from ^{14}C-Entacapone to DNA

non-GLP; Report #[redacted]94031210212; Report date: 12/30/94; Vol: 36

Summary:

In vitro binding of radioactivity to calf thymus DNA was measured after incubation with ^{14}C-entacapone (25-50 µg/mg DNA). No significant binding (<0.01%) was detected after 1 or 24 hr incubations.

C.6.g. Entacapone: Reverse mutation in four histidine-requiring strains of *Salmonella typhimurium* and two tryptophan-requiring strains of *Escherichia Coli* in the presence of carbidopa and L-dopa

GLP; Report #: 544/44-1052 Report Date: 5/29/97 Vol: 36
Conducted by: [redacted]

Summary:

The combination of entacapone and Sinemet, administered at therapeutically relevant ratios (4:4:1 ENT:L-DOPA:carbidopa), was tested for mutagenic affects in the Ames test using the direct plate incorporation method in an appropriate battery of *Salmonella typhimurium* and *E. coli* strains. Top dose selection was based on cytotoxicity. No signs of increased mutant frequency greater than twofold were observed under any test conditions. Positive controls produced the expected results. Thus, the combination of ENT + Sinemet was not mutagenic under the conditions of this Ames test.

Methods:

Drug concs: Entacapone (Batch 008 in DMSO), L-DOPA and carbidopa (both prep'd in 0.1M HCl) were used in a ratio of 4:4:1 (the intended therapeutic ratio). Top dose selection was based on toxicity. A preliminary experiment in strains TA100 and WP2 uvrA indicated only slight toxicity at the limit dose of 5000 µg/plate (combined components; individual E/LD/CD amounts were 2222, 2222, and 556 µg/plate).

Assay: Two experiments were conducted by the direct plate method. In the first experiment, doses were from 8-5000 µg/plate. For the second experiment, the top dose was reduced to 1000 µg/plate for all strains in the absence of S9, and for *E. coli* strains in the presence of S9 because of toxicity in the first assay. The solvent itself was associated with some degree of toxicity in the absence of S9, but because of solubility limitations the amount of solvent was not reduced so that the amount of test article added to the system could be maximized.

Cytotoxicity was not observed in preliminary studies using the preincubation method, so no additional studies were conducted with this method.

Strains: TA 1535, TA 1537, TA 100, TA 98, WP2 pKM101, WP2 *uvrA*pKM101
Pos controls: same as described in the Ames test of ENT alone, except for TA100 (Na azide)
Metabolizing System: S9 fraction prepared from Arochlor 1254-induced rats

Results:

No signs of increased mutation frequency that were greater than twofold compared to control plates were evident under any test condition with the combination or either component alone.

Positive controls produced the expected increase in revertant frequency.

Conclusion: Under the test conditions, the combination of ENT + Sinemet was not mutagenic.

C.6.h. Entacapone: induction of micronuclei in the bone marrow of treated mice in the presence of carbidopa and L-dopa

GLP; Report #: 544/45-1052 Report date: 6/97 Vol: 36
Conducted by: [redacted]

Summary:

The combination of ENT + Sinemte was tested for *in vivo* clastogenic activity in the mouse micronucleus model. Varied ENT doses (40, 200, 1000 mg/kg) were adminsitered by gavage in combination with a fixed dose of Sinemet (40 mg/kg L-DOPA/ 10 mg/kg carbidopa). The ENT high dose was appropriate based on lethality at a slightly higher dose. The basis for Sinemet dose selection was not clear. Animals were sacrificed at 24, 48, and 72 hrs post-treatment.

None of the treatment groups showed evidence of micronuclei formation in bone marrow erythrocytes. No evidence of bone marrow toxicity was observed; thus, the validity of the model was not established.

Methods:

Dosage: Entacapone (Batch 008) was administered by gavage at doses of 40, 200 or 1000 mg/kg (1.2% methylcellulose) in combination with a fixed dose of 50 mg/kg Sinemet (10 mg/kg carbidopa/40 mg/kg L-dopa in 1.2 % methylcellulose). Additional groups were treated with either entacapone alone (1000 mg/kg) or Sinemet alone (40 mg/kg LD/10 mg/kg CD).

Analysis of the dosing suspensions indicated that actual entacapone concentrations were notably greater than nominal concentrations (123-202%).

Cyclophosphamide (80 mg/kg) was the positive control.

The high oral dose selection was based on a preliminary toxicity study in which lethality occurred in 1M and 1F treated with 1200 mg/kg, and 1M treated with 1500 mg/kg.

Animals: CD-1 mice; 20-33 g; 4-5 wks old; N = 5/sex/group;

Animals were sacrificed at 24, 48 and 72 hr post-treatment

Sample Collection and Analysis:

Femoral bone marrow samples were collected, smeared, and at least 2000 PCEs were examined for the presence of micronuclei.

Results:

None of the treatment groups had frequencies of micronuclei that were greater than control levels. The positive control produced the expected result.

The selection of 1000 mg/kg entacapone as the high dose for the study was acceptable based on the findings of mortality at slightly higher doses. The basis for Sinemet dose selection is not clear.

Conclusion:

Entacapone in combination with Sinemet did not induce micronuclei formation in mice under the conditions of the study. The validity of the model was not established by evidence of bone marrow toxicity.

C.7. Carcinogenicity

C.7.a. Entacapone: 104-week carcinogenicity study in mice by gavage

GLP; Report #: [redacted]11353; Report Date: 1/18/96 Vols: 26-28
Conducted by : [redacted]

Summary:

Entacapone was administered by gavage at doses of 20, 100 and 600 mg/kg/day to CD-1 mice (50/sex/dose group, 100/sex/control) for two years. An additional 8/sex/dose were used for plasma level determinations in weeks 26 and 52. Dosage selection was based on saturation of absorption determined in a 13-week range-finding study.

The major treatment-related finding in the study was premature deaths at the highest dose level, most notably in females. Less than 50% of HDF survived past one year, and the group was terminated in week 95, at which point only 9 animals remained. Premature deaths were also significantly elevated in HDM; 14/50 animals survived the entire study duration. A treatment-related cause of death was not established, but a higher incidence of terminal congestion in the lung in HD premature decedents is noted as a possible secondary finding.

No other treatment-related effects on clinical signs, body weight, or food consumption were observed. There were no neoplastic or non-neoplastic lesions that were clearly attributable to entacapone. The NOAEL is considered to be 100 mg/kg.

Plasma levels of entacapone were determined at a single time point (10 min post-dose) in weeks 26 and 52. Based on the finding of similar plasma levels in this study and in the 13-week study, the AUCs determined in the latter study were used to estimate human:mouse exposure ratios at the LD and HD; data were not available for the 100 mg/kg dose level:

Dose (mg/kg)		Mouse AUC (µg.hr/ml)	mouse:human [a]
20	M	1.4	0.1
	F	2.6	0.2
600	M	83	6.9
	F	110	9.2

[a] Human AUC determined by multiplying the AUC of a single dose (1.5 µg.hr/ml) times the highest proposed number of daily doses (10).

The high number of premature HDF decedents, particularly during the early phases of the study, severely limits the amount of information on entacapone's carcinogenic potential over the lifespan of female mice. The fact that the MD was substantially lower than the high dose, and has not been demonstrated to be close to an MTD, prevents the use of this dosage group as a "back-up" to the HD group. Thus, the validity of the female mouse cell of 2-year rodent bioassay model is questionable. Survival in HDM, was also significantly reduced compared to control; the surviving MDM at termination should also have been evaluated histopathologically for this cell to be considered acceptable.

Methods:

Animals: CD-1 mice [redacted] M: 25-35 g, F: 18-28 g
Number: 50/sex/group; 2 controls (Main study); 8/sex/group (TK study)
Housing: Individual
Dosages: 20, 100, 600 mg/kg/day entacapone (Batches 008 & 009)

Dosage selection was based on saturation of absorption as determined in a 13-week range-finding study (doses = 200, 300/600 and 400 mg/kg/day; see review p. 16). In that study, the initial MD of 300 mg/kg/day was increased to 600 mg/kg/day in week 8 due to the absence of toxicity; toxicokinetics determined during week 13 suggested that mouse entacapone exposures (AUC) were similar in the 400 and 600 mg/kg/day treatment groups.

Route: Oral gavage (once daily) in 0.5% methylcellulose (5 ml/kg)

Statistics:

Body weight, food consumption, hematology and organ weight data were analyzed by ANOVA (parametric or nonparametric Kruskal-Wallis depending on variance homogeneity). Survival data were assessed graphically using Kaplan-Meier plots, and pairwise comparisons were made using Wilcoxon rank sum test modified for censored survival data. Histology and tumor data were analyzed using Fisher's Exact Probability Test.

Results:

Mortality:

The distribution of premature deaths among treatment groups was as follows:

	Entacapone (mg/kg/day)				
	C1	C2	20	100	600
M	22/50	26/50	23/50	23/50	36/50*
F	33/50	31/50	29/50	33/50	41/50*

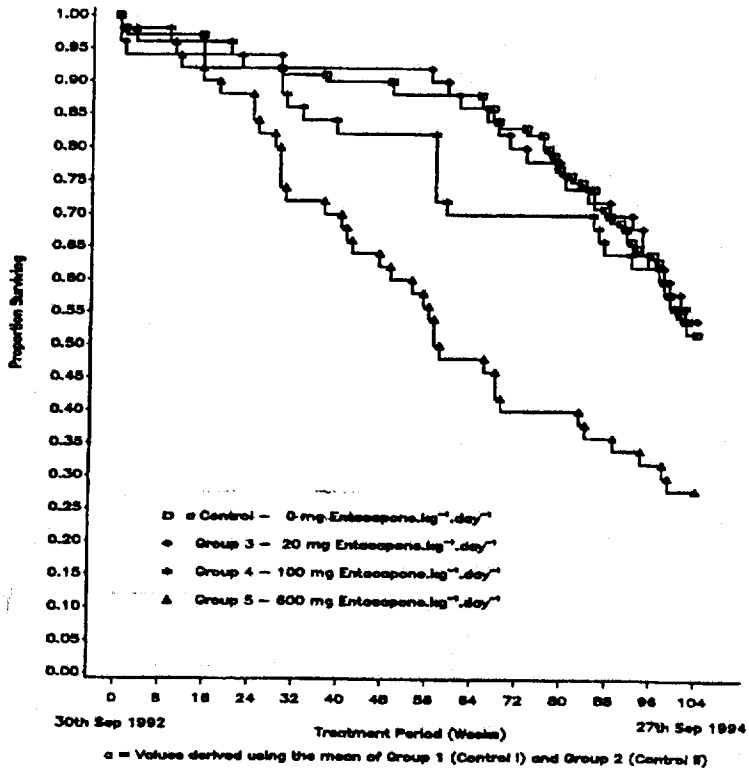
* $p < 0.001$ vs. control

Kaplan-Meier plots (sponsor figures 1&2) show clearly the effect of treatment on survival, particularly in HDF. The most noteworthy findings were the high number of premature decedent HDF after a relatively short treatment period (40% of HDF dead after 4 months; less than 50% survival at one year). Most decedent HDF were found dead as opposed to moribund sacrifices.

No treatment-related cause of death was established.

FIGURE 1

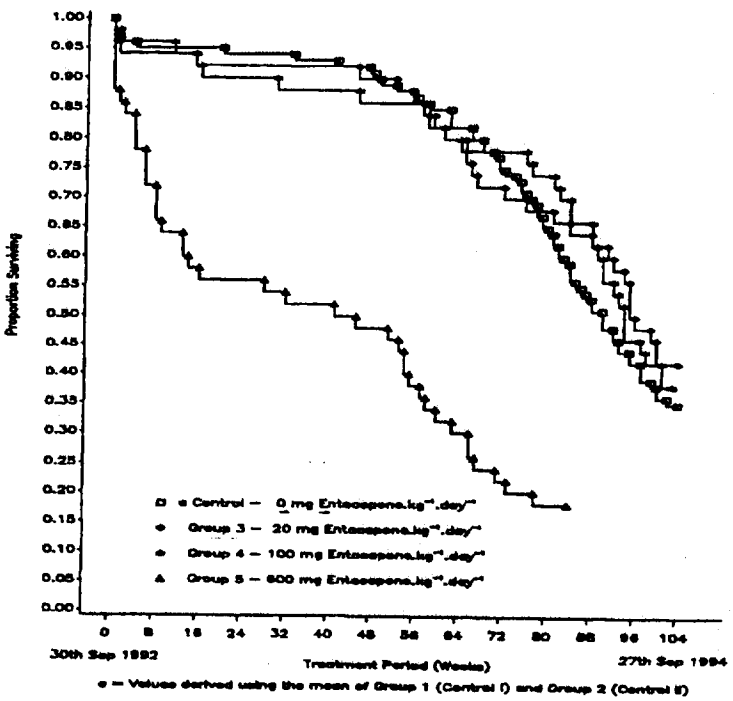
Entacapone
104 Week Carcinogenicity Study in Mice by Gavage
Kaplan–Meier Survival Curve : Males



a = Values derived using the mean of Group 1 (Control I) and Group 2 (Control II)

FIGURE 2

Entacapone
104 Week Carcinogenicity Study in Mice by Gavage
Kaplan–Meier Survival Curve : Females



a = Values derived using the mean of Group 1 (Control I) and Group 2 (Control II)

Clin Obs: Yellow staining if body surfaces and bedding (i.e., urine) at MD and HD.

Body Wt: No treatment-related effects (Sponsor Figs. 3 & 4).

Food/
Water Cons: No treatment-related effects.

Hematology: The only statistically significant difference among groups at termination was a reduction in Hb in MDF. The sponsor did not consider this treatment-related since HDF means were not decreased. However, any group data from HDF survivors may be biased by the high group mortality. Moreover, the group mean data were expectedly highly variable due to (likely) incidental conditions of individual animals.

Blood Smears: Slides from 10 animals/group were analyzed, and revealed no treatment-related changes in red cell morphology.

Organ Wts: No treatment-related ~~effects~~.

Mean ovary weights of MDF were nearly twice as high as control, but the data were highly variable and not associated with any gross path findings.

Gross Path: The type, group distribution, and incidence of necropsy findings provided no evidence of a treatment-related effect.

Histopath: Complete analyses were conducted on all control and HD animals, MDF, and premature decedents. A significant degree of autolysis occurred with HDF tissues that compromised diagnoses in ~~this group~~.

Non-neoplastic findings

Terminal kill animals: The type, group distribution, and incidence of necropsy findings did not provide substantial evidence of a treatment-related effect.

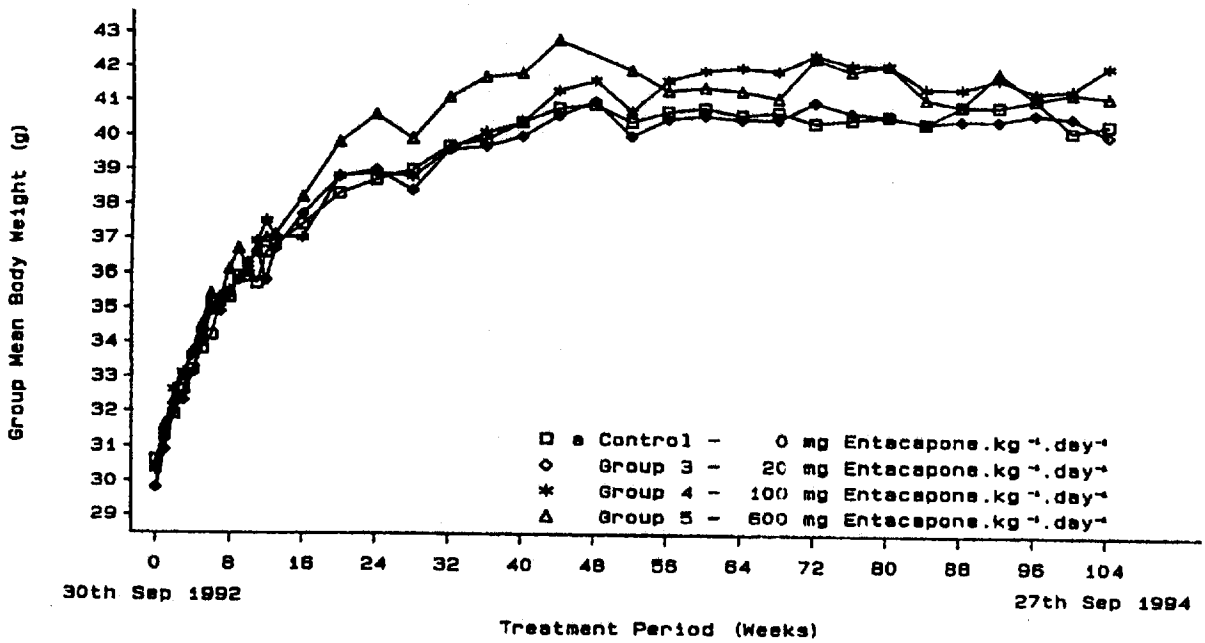
Premature decedents: The only finding with a pattern of occurrence suggesting a potential treatment relationship was terminal congestion in lung:

	Con	LD	MD	HD
M	2/48			11/36
F	2/64	1/29	3/31	21/50

The sponsor did not comment on this finding and its possible relationship to treatment. Although this type of finding is generally considered somewhat nonspecific decedent animals, the dose-relationship is interesting and raise the possibilities that the finding is not simply an agonal event, but may be secondary to the (unknown) treatment-related mode of death.

FIGURE 3

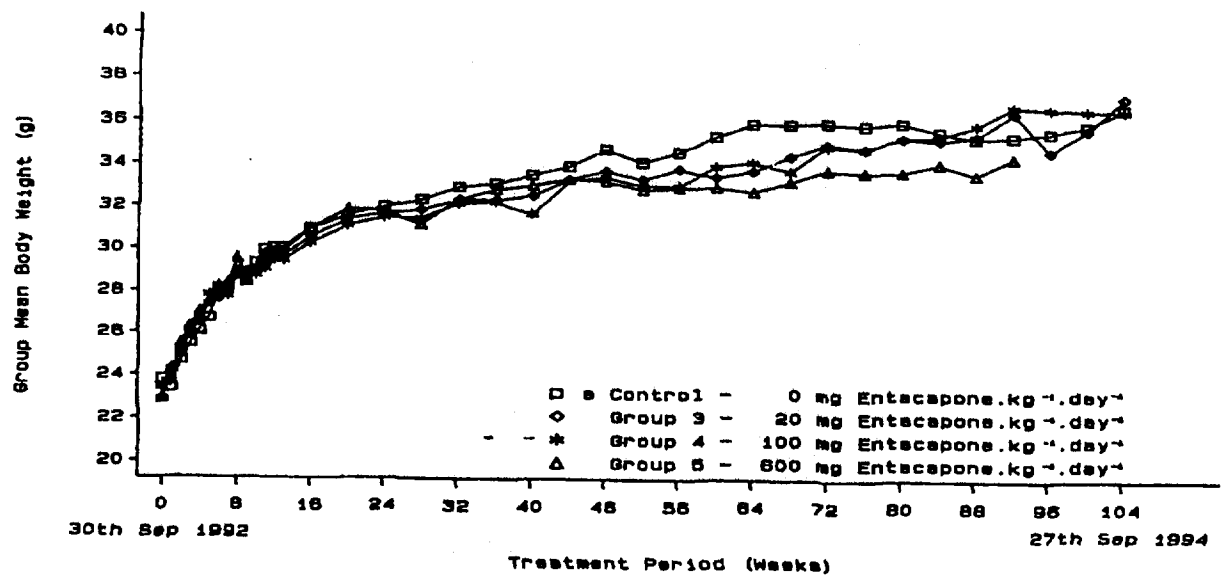
Entacapone
104 Week Carcinogenicity Study in Mice by Gavage
Group Mean Body Weight (g): Males



a = Values derived using the mean of group 1 (Control I) and Group 2 (Control II)

FIGURE 4

Entacapone
104 Week Carcinogenicity Study in Mice by Gavage
Group Mean Body Weight (g): Females



a = Values derived using the mean of group 1 (Control I) and Group 2 (Control II)

Neoplastic findings

The sponsor's analysis of the tumor incidence data revealed no notable neoplastic findings that could be attributable to entacapone in either sex. The Agency's statistical review (Appendix 1), which also considered the intercurrent mortality rate, also did not reveal any significant increases in tumor incidence related to entacapone administration. Both analyses were conducted with pairwise comparisons since not all groups were analyzed completely.

Plasma Concs:

Plasma levels of entacapone were determined in satellite animals (n = 4/sex/group) at 10 min and 24 hr postdose during week 26 and 52. No useful data was obtained at 24 hr. A possible gender difference was noted (levels higher in F vs. M):

$C_{10\,min}$ (µg/ml)

		20	100	600
Wk 26	M	1.7	12.0	39.7
	F	10.5	47.1	74.0
Wk 52	M	2.0	47.1	50.3
	F	9.9	35.9	79.9

The peak levels after the 600 mg/kg dose were generally similar to those observed at week 13 of the subchronic study.

C.7.b. Entacapone: 104 week carcinogenicity study in rats by gavage

GLP; Report #: 11312; Report Date: 1/31/96 Vols: 29-30

Conducted by :

Summary:

Entacapone (20, 90, 400 mg/kg/day) was administered by gavage to Crl:CD rats (50/sex/dose group, 100/sex/control) for two years. The basis for dose selection was not clearly specified, but a 13% reduction in body weight gain was observed in the 13-week study with 400 mg/kg/day in males and females. Toxicokinetic analyses were conducted in satellite animals (5/sex/dose; wks 4, 26, 52, 78, 104).

Survival was not affected by entacapone. Body weights were slightly reduced in HDM (7.4%), and less so in HDF. Slight decreases in RBC parameters were evident in MDM and HDM at termination.

The most notable non-neoplastic and neoplastic lesions were in kidney. Focal tubular epithelial hyperplasia was observed in 3 HDM and 1 LDF. Benign tubular adenomas were seen in 6 HDM. Malignant tubular carcinomas were found in 5 HDM and 1 ConM, and also in 1 LDF and 1 HDF. The incidence of combined adenomas and carcinomas was statistically significant in HDM according to the Agency's analyses. Analysis of tumor incidence in females yielded no statistically significant findings.

The sponsor proposes that the rat renal tumors associated with entacapone administration are male rat specific, and thus possibly related to alpha$_2$-microglobulin deposition (α2-μG). Alterations in the renal handling of this male-rat specific protein is an established mechanism of renal tumorigenesis, and not considered relevant to humans. The evidence for this mechanism is reviewed and evaluated in section C.7.c., and should also be considered by the CAC-EC.

The only other potential treatment-related histopathological finding was ulcerated lesions of the stomach in males; however, this finding occurred at a high background incidence in females, and may be incidental. Chronic progressive nephropathy was observed at similarly high incidences in control and treated animals.

Toxicokinetic analyses suggested that increases in plasma exposures were approximately dose-proportional, and generally similar over the course of the study. Relative to estimated plasma exposures in humans receiving the maximum recommended daily dose (200 mg up to 8 times daily; AUC = 12 μg.hr/ml), entacapone exposures in male and female rats were:

	Males	Females	
LD:	0.9 - 2.9	1.6 - 3.6	times the human exposure
MD:	4.9 - 5.6	4.1 - 9.1	"
HD:	10.0 - 31.6	16.6 - 29.1	"

The NOAEL in males was 20 mg/kg/day. Because of the equivocal renal findings in females, the NOAEL was not clearly established.

The male arm of the study was a valid assessment of the carcinogenicity of entacapone, as survival was

adequate, and the high dose of 400 mg/kg/day was at or near the MTD based on body weight reduction and tumor formation. The absence of clear clinical signs of toxicity or histopathologies in females raises the concern that the high dose was not an MTD in this study. However, based on the observation of reduced body weight gain in the 13-week study, and the slightly increased mortality in HDF, 400 mg/kg appeared to be sufficiently close to an MTD in females.

Methods:

Animals: Crl:CD rats (Charles River, UK); M: 181-266 g, F: 121-183 g; 6 wks
Number: 50/sex/group; 2 controls (Main study); 5/sex/group (TK study)
Housing: Group (5/cage)
Dosages: 20, 90, 400 mg/kg/day entacapone (Batches 008 & 009)

The sponsor did not clearly specify the basis for dose selection, but indicated that it was based on results of 28 day, 13 and 52 weeks studies.

Route: Oral gavage (once daily) in 0.5% methylcellulose (5 ml/kg)

Statistics:

Body weight, food consumption, hematology and organ weight data were analyzed by ANOVA (parametric or nonparametric Kruskal-Wallis depending on variance homogeneity). Survival data were assessed graphically using Kaplan-Meier plots, and pairwise comparisons were made using Wilcoxon rank sum test modified for censored survival data. Histology and tumor data were analyzed using Fisher's Exact Probability Test.

Results:

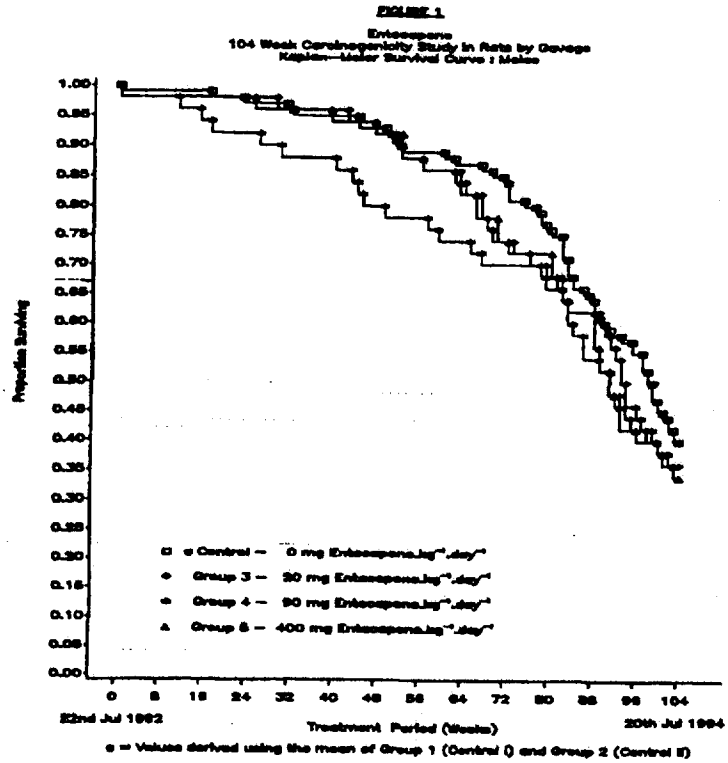
Mortality:

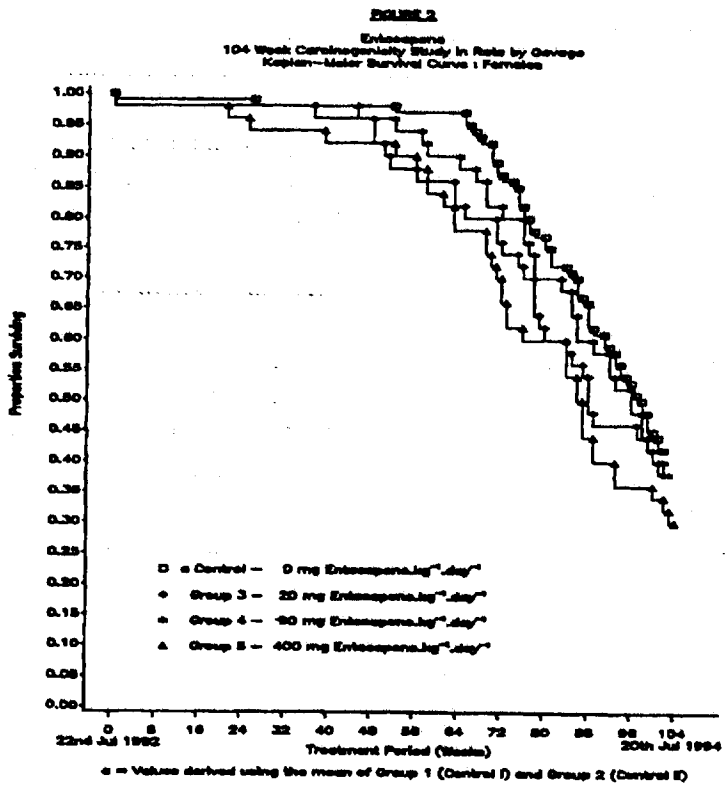
Overall group mortality rates were not significantly affected by entacapone, as shown in a table of the distribution of premature deaths and in Kaplan-Meier plots (sponsor figures 1&2).

The only deaths that were considered attributable to entacapone were 3 HDM with malignant renal tubular carcinomas.

Mortality Incidences

	C1	C2	Entacapone (mg/kg/day)		
			20	90	400
M	34/50	26/50	32/50	32/50	33/50
F	31/50	27/50	32/50	31/50	35/50





Clin Obs: Salivation in MD and HD. Staining of body surfaces and cage trays was evident in all treatment groups.

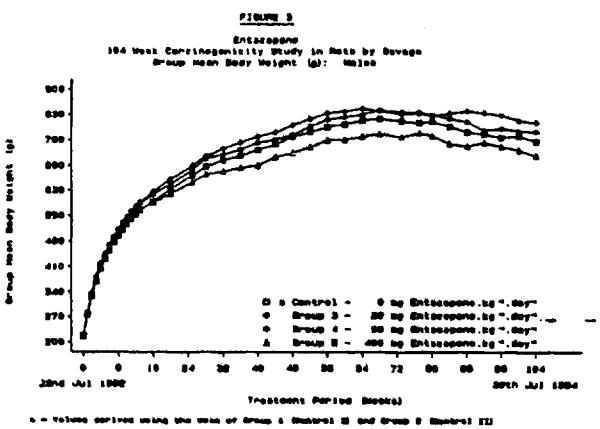
Body Wt: Terminal body weights were reduced in HDM and to a lesser extent in HDF. The body weight time course profiles (sponsor Figures 3 & 4) suggest a relatively consistent suppression of 5-6% in HDM and 7-9% in HDF over latter portions of the study (Note: the terminal weight for HDF in Sponsor Fig.4 is not consistent with that shown in the sponsor's summary or individual tables, which are assumed to be the correct values).

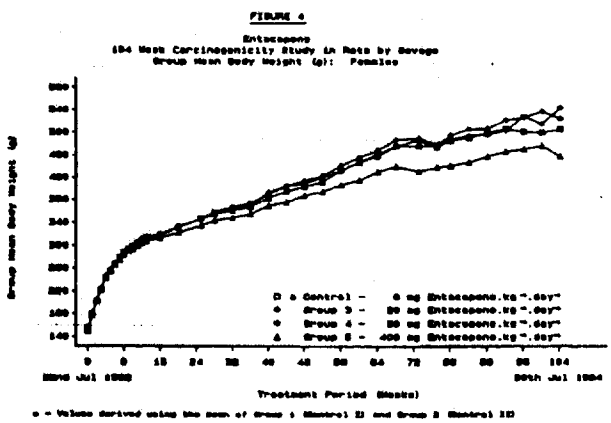
MALES

	WK 0	WK 104	% CON
Control	218	775	-
20 mg/kg	220	809	104.4
90 "	218	784	101.2
400 "	215	718	92.6

FEMALES

	WK 0	WK 104	% CON
Control	155	507	-
20 mg/kg	151	526	103.7
90 "	149	546	107.7
400 "	149	490	96.6





Food/
Water Cons: No treatment-related effects.

Hematology: Slight, treatment-related decreases in erythrocyte parameters were evident in males at termination. Statistically significant group mean variations were:

↓ Hb	-	MDM, HDM
↓ RBCs	-	HDM
↓ Hct	-	MDM, HDM
↓ MCH	-	MDM
↓ MCV	-	MDM

Blood Smears: Slides from 6-10 animals/group were randomly selected analyzed. No treatment-related changes in red cell morphology were identified.

Organ Wts: The following changes were noted, but not considered by the sponsor as toxicologically meaningful due to either the small magnitude, lack of statistical significance (n.s.), or absence of corresponding gross or histopathological findings:

↑ kidney (rel)	-	HDM (18%, n.s.), HDF (9%, n.s.)
↑ heart (rel)	-	MDM (9%), HDM (10%)
↓ testes (rel)	-	HDM (16%)
↑ ovaries (rel)	-	HDF (212%, n.s.)

Gross Path: Kidney masses were present in 5/50 HDM as compared to 2/100 control animals. Staining of the skin/subcutis was evident in 17/50 HDM, and 7/50 HDF as compared to 3/100 ConF and ConM.

Histopath: Complete analyses were conducted on all control group 1 and HD animals, and premature decedents. Due to the increased incidence of renal epithelial tumors in HDM, the kidneys of all animals were evaluated histologically.

Non-neoplastic findings

Focal tubular epithelial hyperplasia in the kidney was seen in 3 HDM and 1 LDF. In view of the apparent treatment-related increase in renal neoplasia (see below), this finding is considered as likely related to treatment.

The only other finding that occurred at a higher incidence in treated animals relative to controls was ulcerated lesions of the stomach in males (1/76 Con, 2/31 LD, 3/32 MD, 6/50 HD). However, this finding was observed in 6/77 control F; thus, its treatment-relationship is equivocal.

Neoplastic findings

The distribution and type of renal epithelial tumors among treatment groups were as follows:

		C1	C2	20	90	400
tubular adenoma (B)	M	0/50	0/50	0/50	0/50	6/50
	F	0/50	0/50	0/50	0/50	0/50
tubular carcinoma (M)	M	0/50	1/50	0/50	0/50	5/50
	F	0/50	0/50	1/50	1/50	0/50
combined	M	0/50	1/50	0/50	0/50	11/50
	F	0/50	0/50	1/50	1/50	0/50

Malignant tubular carcinoma was identified as the probable cause of death or premature sacrifice of 3 HDM and 1 MDF (the histopathologist also refers to this tumor as a clear cell adenocarcinoma). Benign adenoma was indicated a probable cause of premature sacrifice for 1 HDM.

Only 1 tumor-bearing HDM had coexisting focal tubule hyperplasia. The LDF with tubular carcinoma was not the same LDF with tubule hyperplasia, and had no other renal histopathology finding. All tumor-bearing HDM, except for two animals with adenomas, had a coexisting diagnosis of chronic nephropathy with severities ranging from very mild to severe. Specific pathological characteristics of chronic nephropathy were not defined. This finding was evident at similarly high incidences in all groups, but more prevalent in males versus females (73 ConM, 38 LDM, 38 MDM, 43 HDM; 25 ConF, 14 LDF, 19 MDF, 10 HDF).

The sponsor's analysis of the tumor incidence data identified only tubular adenoma in males as a statistically significant finding ($p < 0.05$; Fisher's Exact test between HD and control). According to the Agency's statistical review (conducted by Roswitha Kelly, Division of Biometrics I; see Appendix 1), the sponsor's analysis was a two-sided test. Using a one-sided mortality adjusted pairwise permutation test in her review, Ms. Kelly found that both adenomas and carcinomas were statistically significant in HDM ($p = 0.0051$ and $p = 0.0270$, respectively). When adenomas and carcinomas were combined, the p-value was 0.0006.

The determination of whether the carcinoma is considered a common tumor should be addressed by the Center's CAC. Factors to be considered should include the occurrence of this tumor type in concurrent controls and historical data, some of which have been provided by the sponsor (Appendix 2). A compilation of data from ten 104-week rat gavage bioassays (apparently) conducted at the sponsor's contract facility (Inveresk) showed the following incidences of renal findings in a total of 494 male rats and 492 female rats (strain not specified):

benign (non-lipomatous) tumor:	1 M (0.2%)
malignant tumor:	1 M (0.2%)
focal hyperplasia:	2 F (0.4%)

A data compilation generated by the animal supply facility (Charles River) of renal tumors identified in nineteen 24-month studies in CD rats was also provided (1253 males, 1258 females). The number of lesions and the incidence rates were as follows:

renal cell adenoma:	3 M (0.24%); 1 F (0.08%)
renal cell carcinoma:	3 F (0.24%)
renal cell adenocarcinoma:	4 M (0.32%)
tubular hyperplasia:	9 M (0.72%); 13 F (1.03%)

Based on these historical data, the occurrence of renal tumors in 24-month rat bioassays is a relatively rare event, which leads to an obvious conclusion that the presence of renal tumors in entacapone-treated males is related to treatment. Less obvious is whether the carcinomas in females were treatment-related, since no HD animals were affected. Therefore, the issue that needs to be considered must address the relative rarity of the tumors versus the absence of a dose-relationship. This determination is important in this instance to an overall evaluation, since the sponsor posits that the renal tumors are a male rat-specific phenomenon with no relevance to humans.

Ms. Kelly notes in her review that the statistical significance of the findings would have been greater using the more powerful trend test, but this test was not conducted due to time constraints.

Neither the sponsor nor the Agency reviewer found any other statistically significant incidences of neoplasia related to entacapone. The reviewer's survey of the data also did not identify any additional potentially treatment-related neoplastic findings.

Plasma Concs:

Plasma levels of entacapone were determined in satellite animals (n = 5/sex/dose) at 15 min, 4, 8, 12, and 24 hr postdose during day 1 and weeks 26, 52, 78 and 104. The AUC data and the relative human exposure factors are presented in the following table:

Dose (mg/kg/day)	Week	AUC (μg.hr/ml)		Exposure factor [1]	
		M	F	M	F
20	26	11	19	0.7	1.3
	52	27	36	1.8	2.4
	78	34	44	2.3	2.9
	104	24	37	1.6	2.5
90	26	62	50	4.1	3.3
	52	67	110	4.5	7.3
	78	64	100	4.3	6.7
	104	58	89	3.9	5.9
400	26	120	350	8.0	23.3
	52	290	260	19.3	17.3
	78	380	320	25.3	21.3
	104	130	200	8.7	13.3

[1] Exposure factor was calculated by dividing AUC_{rat} by AUC_{man}. The AUC_{man} was obtained by multiplying the AUC of entacapone after a single dose (1.5 μg.hr/ml) with the maximum dosing frequency (i.e. 10).

C.7.c. Mechanistic Studies of Rat Renal Tumor Formation

The sponsor posits that the occurrence of renal tumors in the rat 2-year bioassay is a male rat-specific phenomenon, possibly related to the accumulation of alpha-2 microglobulin (α2-μG), a protein that is not present in females rats, mice or humans. Obviously, demonstrating the involvement of a male rat-specific event or pathway in renal tumor formation would diminish the potential human relevance of the carcinogenicity study finding, and have significant regulatory implications (i.e., product labeling). In Appendix 67 (volume 31), the sponsor has prepared an overview of evidence from some of their standard toxicology studies that suggests that renal histopathologies and tumorigenesis occurred only in male rats. Subsequently, the sponsor conducted some additional histological and immunocytochemical studies to characterize the lesions and more conclusively identify α2-μG in the specimens. The findings will be described and evaluated in this section.

1. Rat 104-week Bioassay (H/E stained sections): As described in the preceding section, the incidence of renal tubule adenomas and carcinomas was significantly higher in male rats treated with 400 mg/kg/day entacapone (HDM). In addition, 2 non-tumor-bearing HDM and 1 of the tumor-bearing HDM had tubule hyperplasia.

   At least two lines of evidence suggest that renal neoplasia may not be solely attributed to the α2-μG male rat specific pathway:

   - A low incidence of renal carcinomas (1 LDF, 1 MDF) and tubule hyperplasia (1 LDF), relatively rare rat histopathological findings according to historical databases provided by the sponsor, was observed in entacapone-treated females. The absence of a dose-response relationship raises the possibility that these findings were incidental.

   - No additional specific pathological features that are generally characteristic of α2-μG deposition were reported by the pathologist (eg. hyaline droplet deposition, necrosis, granular casts, linear mineralization of renal papilla). The only coexisting pathology reported was ill-defined chronic nephropathy (very mild to severe), which occurred at a similarly high incidence in all treatment groups including controls.

     According to the sponsor, hyaline droplet deposition declines progressively after 18 months of age, which could account for its absence from this study. However, Hard et al. (1993) reported hyaline droplets (and the aforementioned changes) as a consistent finding in 2-year bioassays with eight compounds that induce α2-μG renal tumors.

2. 28-day Rat Study (Appendix 48, Vol. 12:): Rats (n = 10/sex/group) were treated with 15, 95 or 600 mg/kg/day. Sections were stained with hematoxylin-van Geisson. All HDM that survived the study (7/7) and 3/10 ConM exhibited hyaline bodies in the tubular epithelium. Hyaline bodies were not observed in any females or LD or MD groups.

   The initial conclusion of the study pathologist was that the "tissue sections did not reveal any changes related to the treatment with OR-611...", and that there were "...no changes indicating severe or irreversible organ or tissue damage". In the Overview, the sponsor's revised assessment

states that hyaline droplets were seen at an increased incidence in entacapone-treated males. This conclusion is reasonable, and may have been initially discounted since hyaline droplets were not observed in any LD or MD males. Hyaline droplets containing α2-μG are generally present in normal male rats, but compounds that bind α2-μG can form a poorly digestible complex that exacerbates the appearance of hyaline droplets.

It is also noted in this study that erythrocytes appeared in urine of HDF and HDM.

3. Histological Studies of Rat Kidneys Treated for 6 Weeks with Entacapone (Appendix 68, Vol.31): Sections of renal tissue were obtained from Con or ENT-treated animals (n = 6/sex; ENT dose = 500 mg/kg twice daily for 6 weeks in males, or 6-8 weeks in females) during the preliminary Seg I/Seg III study. Sections were stained with either Martius Scarlet Blue to detect α2-μG or PAS to highlight the brush border of the PCT.

ENT-treated males displayed staining consistent with α2-μG deposition (granular, MSB positive; PAS disruption of brush border).

ENT-treated females showed nuclear and cytoplasmic degenerative changes (basophilic tubules, karyomegaly, nephrosis), but no proteinaceous droplets.

4. A Special Stain for Protein in the Kidneys of Rats Treated for 52 Weeks with Entacapone: Kidney sections from all male rat groups (0, 20, 90, 400 mg/kg/day), Con F, and HDF in the 52-week study (n = 20/group) were re-examined following Martius Scarlet Blue staining for protein (eg., fibrin, hyaline). Additional sections from control and HD males and females were cut and stained with H&E.

The slides were not blinded for the study pathologist.

All animals were positive for intracytoplasmic hyaline protein droplets, but HDM differed from ConM with respect to staining intensity ($p < 0.001$), anatomic region (P3 in some HDM; P1-P2 in others), and protein appearance (granular characteristic of α2-μG binding in 9/20 HDM vs. 2/20 ConM).

Treated and control females did not differ with respect to these parameters.

Other renal findings that were uncovered during this re-evaluation, and not present during the initial evaluation, are discussed within the main study summary (see p. 31). Briefly, chronic progressive nephropathy and enlarged nuclei were evident at a higher incidences in HDM. The incidence of enlarged nuclei was increased slightly in HDF (14/20 vs. 8/20 in Con F).

5. Immunocytochemical Staining to Identify α2-μG in Rat Renal Tissue
Conducted by [redacted]
Report #s: 95/ORP048/0300 & 95/ORP050/0500)

Kidney sections were collected from animals treated with entacapone in the preliminary Seg I/III study (#3 above; n = 6; doses = 0, 500 mg/kg, b.i.d. for 6-7 weeks) and in the 52-week study (#4

above; n = 20; doses = 0, 20, 90, 400 mg/kg/day). Sections were stained with either H&E or a monoclonal antibody for rat α2-μG for immunocytochemical analysis.

The data from the immunocytochemical staining of male rat kidney sections from the Seg I/III study are shown in the Sponsor's Table. Staining in the pars convoluta was similar in control and treated animals. Staining scores in the pars recta and descending loop of Henle tended to be higher in treated animals versus controls.

Table 1 — Immunocytological Staining of Rat Kidneys

Animal Number	1	2	3	4	5	6	-ve Control	female
Proximal tubules								
pars convoluta	2	2	2	2	2	1	0	0
pars recta	2	1	1	3	2	1	0	+/-
Descending loop of Henle	1	1	0	1	0	0	0	0

Animal Number	19	20	21	22	23	24	+ve Control
Proximal tubules							
pars convoluta	2	2	2	2	2	2	2
pars recta	2	3	2	3	3	3	3
Descending loop of Henle	0	2	1	1	2	2	2

0 - No staining
1 - Minimal staining
2 - Slight staining
3 - Moderate staining

+/- Some very minimal non-specific staining

The positive and negative controls were not specified.

The immunocytochemical data from the 52-week study are shown in the Sponsor's table. Results were generally comparable to the SegI/III study in that the pars recta appeared as the region showing the largest difference in α2-μG staining intensity.

Table 1 — Immunocytological Staining of Rat Kidneys

	Animal number	51	52	53	54	55	56	57	58	59	60	61	62	63	64	65	66	67	68	69	70
Con	Proximal tubules pars convoluta	3	2	3	2	3	2	2	2	2	2	2	2	2	3	2	3	2	2	1	2
	pars recta	1	1	1	1	2	1	1	1	1	1	1	1	1	2	1	2	1	1	1	1
	Descending loop of Henle	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0
	Animal number	151	152	153	154	155	156	157	158	159	160	161	162	163	164	165	166	167	168	169	170
LD	Proximal tubules pars convoluta	3	3	3	3	3	2	2	3	3	3	3	2	3	2	3	2	2	2	3	2
	pars recta	2	1	2	1	1	1	1	1	2	1	1	1	1	1	1	1	1	1	1	1
	Descending loop of Henle	0	0	0	0	0	0	0	0	1	0	0	0	0	0	1	0	0	0	0	0
	Animal number	251	252		254	255	256	257	258	259	260	261	262	263	264	265	266	267	268	269	270
MD	Proximal tubules pars convoluta	2	2		2	2	2	2	2	2	2	2	2	2	2	3	2	2	2	2	2
	pars recta	2	2		1	2	3	1	2	2	1	2	1	2	1	2	2	1	2	1	1
	Descending loop of Henle	0	0		1	0	0	0	1	0	0	0	0	0	0	1	0	0	0	0	0
	Animal number	351	352	353	354	355	356	357	358	359	360	361	362	363	364	365	366	367	368	369	370
HD	Proximal tubules pars convoluta	2	2	3	3	3	3	3	2	3	3	2	2	2	2	2	2	2	3	1	2
	pars recta	3	3	3	2	2	2	3	3	3	3	2	3	2	3	2	3	2	3	0	3
	Descending loop of Henle	0	0	0	1	0	1	2	1	0	1	0	0	0	0	0	0	1	0	0	1

0 - No significant staining
1 - Minimal staining
2 - Slight staining
3 - Moderate staining

The sponsor concludes that their compilation of data "strongly suggest that the mechanism behind the tubular tumors in male rats found in the carcinogenicity study with entacapone is related to abnormal behavior of α2-μG in the male rat kidneys."

In the Discussion section of both immunocytochemistry reports, the contract pathologist concludes that the relevance of the variations in staining intensity are equivocal, mainly because of the absence of associated pathological changes that are typically seen in α2-μG nephropathy (necrosis, epithelial cell proliferation, granular casts, linear mineralization of the renal papilla, urothelial hyperplasia). However, the potential involvement of α2-μG is not completely discounted (by the pathologist) since some of the changes may become less apparent with age, and since α2-μG tends to increase the onset and severity of chronic progressive nephropathy, as was observed in the 52-week study (an actual effect on onset is not established by the data).

In the opinion of the reviewer, the compilation of data provided by the sponsor does is not adequate to wholly attribute to the rat renal tumors to altered α2-μG handling for the following reasons:

1. The inconsistencies between the entacapone-induced renal changes and the "typical" case of α2-μG nephropathy, as cited by the sponsor's contract pathologist.

2. Much of the data that the sponsor views as support for the α2-μG hypothesis apparently was collected *post hoc*, and in at least one study (52-week histological re-evaluation) the pathologist was not blinded. One conclusion from this set of circumstances is that the data (i.e., kidney tissues) were re-evaluated by the sponsor after they learned of the male rat renal tumors in search for support for the α2-μG male rat-specific pathway. Obviously, this raises the concern of whether the data analyses were unbiased.

3. Two renal carcinomas (1 LDF, 1 MDF) and 1 case of tubular hyperplasia (1 LDF) were found in females. While these incidences are low, and not dose-related, the spontaneous occurrence of these lesions is relatively rare. Although the changes cannot be conclusively ascribed to entacapone, their occurrence casts some doubt on the suggestion that entacapone-induced renal tumorigenesis is an exclusively male rat-specific event.

4. The closely-related (chemically and pharmacologically) compound tolcapone (TASMAR) also produced renal tumors (carcinoma: 1 MDM, 3 HDM, 1 HDF; adenoma: 2 MDF, 1 HDF). The sponsor of TOL did not propose or investigate the involvement of α2-μG in the tumorigenic effects of tolcapone, since a broad spectrum of extensive and severe non-neoplastic degenerative renal changes were observed in TOL-treated male and female rats, which led to the suggestion of a regenerative hyperplasia/neoplasia mechanism, possibly as a result of metabolic overload. Thus, the two compounds may cause renal tumors by different mechanisms. However, considering the combined findings of tumors in entacapone-treated females and the chemical and pharmacological similarities of tolcapone and entacapone, the possibility that other renal events unrelated to α2-μG deposition may be involved in the effect of entacapone can not be excluded.

5. Entacapone was positive in two *in vitro* clastogenicity assays; the validity of the in vivo micronucleus test (bone marrow erythrocytes) was questionable by the absence of bone marrow toxicity.

D. PHARMACOKINETIC/ADME STUDIES

D.1 Single Dose Pharmacokinetics/Absorption
- a. Rats
- b. Dogs

D.2 Distribution
- a. Tissue distribution in rats
- b. Protein binding and displacement

D.3. Metabolism
- a. Rat plasma
- b. Rat urine and bile
- c. Dog urine
- d. Comparison of metabolite profiles of experimental animals and man

D.4. Excretion
- a. Rat urinary and fecal excretion
- b. Rat biliary excretion
- c. Rat milk excretion
- d. Dog urinary and fecal excretion

D.1. Single-Dose Pharmacokinetics/Absorption

D.1.a. Rats

The single-dose pharmacokinetics of entacapone were determined in rats (Han:Wistar or Crl:CD) after oral (10, 65, 400 mg/kg by gavage in 1.2% methylcellulose) or intravenous (5 mg/kg) administration.

Absorption after oral administration was rapid, and bioavailability was low to moderate (dose-dependent, but the estimates were derived using only a single i.v. dose level). A second peak was consistently noted suggesting the possibility of reabsorption from the intestine. Greater than dose proportional increases in AUC were evident possibly indicating a saturation of first-pass metabolism. The increase in Cmax was less than dose proportional possibly due to saturation of absorption. Elimination half-lives were short, and the volume of distribution small. Isomerization to Z-isomer was minimal (< 2%). Repeated oral dosing (10 mg/kg for 7 days) did not result in drug accumulation.

Single-Dose Entacapone Pharmacokinetics in Rats

Route	Dose (mg/kg)	t_{max} (hr)	C_{max} (µg/ml)	$AUC_{(0\text{-}inf)}$ (h.µg/ml)	V_{ss} (l/kg)	Cl_{tot} (l/hr/kg)	$t_{1/2}\alpha$ (hr)	$t_{1/2}\beta$ (hr)	F*
oral (1.2% MC)	10	0.1	5.6	3.7					18
	65	"	30.4	58.3					44
	400	"	97.2	444.3					54
i.v.	5			10.1	0.19	0.5	0.11	0.44	

n = 7-8/group

D.1.b. Dogs

The total absorption of entacapone-related materials was assessed in a radiolabel study in dogs (20 mg/kg in capsule or 5 mg/kg I .v. ^{14}C-entacapone). Unlabelled entacapone was used in a dose-dependency study (20, 40 80 mg/kg, p.o. in capsule).

Entacapone was absorbed rapidly in dogs with a moderate bioavailability. The percentage of the entacapone dose systemically available as unchanged compound and its (Z)-isomer relative to the degree of total absorption suggests moderate first pass metabolism.

Entacapone plasma concentrations declined rapidly. The half-life of radiolabel was about 8-fold greater than that of parent compound, but counts were near or at background levels 24 h after radiolabel administration. The i.v. bolus dose of 5 mg/kg was initially distributed to a rather small central volume, and the steady state distribution was also small. This finding is consistent with the physicochemical properties of entacapone (pKa is 4.5, logD at pH 7.4 is -0.2), and its extensive protein binding (about 90 % in dog). Systemic clearance (about 26 ml/min/kg) was slightly lower than the hepatic blood flow in the dog (about 40 mi/mm/kg).

More substantial isomerization to the Z-isomer was seen in dogs (~ 20%) as compared to rats (<2%).

The higher radioactivity levels in plasma as compared to unchanged parent and the Z-isomer indicates the presence of the metabolites, which were identified in plasma samples taken 0.5 and 2 hours after oral administration. Besides entacapone and the Z-isomer, the 0-glucuronides of entacapone and its (Z)-isomer accounted about 30 % of the radioactivity in 2-h plasma.

A repeat dose oral PK study in dogs indicated little or no entacapone accumulation after 7 days of treatment with 20, 40 or 80 mg/kg (accumulation factors of 0.9 - 1.3)

Single-Dose Entacapone Pharmacokinetics in Dogs

Radiolabel Study

Route	Dose (mg/kg)	Analyte	t_{max} (hr)	C_{max} (µg/ml)	$AUC_{(0\text{-inf})}$ (h.µg/ml)	V_{ss} (l/kg)	Cl_{tot} (l/hr/kg)	$t_{1/2}\alpha$ (hr)	$t_{1/2}\beta$ (hr)	F
oral	20	E	1.8	2.7	4.7				0.6	29
		Z	2.0	0.7	1.3				0.7	8
		^{14}C	2.0	6.9	22.7				3.0	50
i.v.	5	E			3.5	0.4	1.5	0.12	0.45	
		Z			0.5				0.33	
		^{14}C			11.3		0.45		3.7	

n = 2M & 2F

Dose-Dependency Study

Route	Dose (mg/kg)	Analyte	t_{max} (hr)	C_{max} (µg/ml)	$AUC_{(0\text{-inf})}$ (h.µg/ml)	$t_{1/2}$ (hr)	F
oral	20	E	1.3	3.4	4.8	0.4	30.1
		Z	1.3	0.6	1.2	0.6	7.3
	40	E	1.5	5.1	11.4	0.8	35.4
		Z	1.5	1.1	2.9	1.0	8.9
	80	E	1.3	9.6	23.6	0.9	36.7
		Z	2.0	1.8	5.9	1.0	9.2

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D.2. Distribution

D.2.a. Tissue distribution in rats

Tissue distribution of the total radioactivity was investigated in rats after single and 7-day repeated oral dosing (10 mg/kg; 1 mg/kg ^{14}C-entacapone; 15 µCi/rat; n = 3 animals per time point), or single i.v. dosing (3 mg/kg ^{14}C-entacapone; 19 µCi/rat). Tissues were sampled at 1 and 24 hours following oral dosing, and at 0.25, 1, 3, and 24 hours following an intravenous bolus dose. Total radioactivity in tissues, urine and feces (collected quantitatively) was determined by LSC after sample oxidation.

Generally comparable tissue distribution profiles were observed after single and repeated oral dosing with ^{14}C-entacapone, mainly restricted to organs of absorption and elimination. At 1 hr post-dose, highest levels were in the GI tract (~65%) and urine (12-18%). Detectable levels (<5%) were in the blood, plasma, liver, kidneys, fat, skin, and skeletal muscle. At 24 hr, radioactivity was restricted to GI tract (<10%), urine (~30%), and feces (55-70%). Essentially no radioactivity was present in brain after either oral regimen indicating poor BBB penetration (brain:plasma ratio ≤ 0.01). The similarity in profiles after single and repeated oral dosing suggests that tissue accumulation does not occur.

After intravenous administration, highest levels were again in the GI tract (35-60%; increase with time up to 3 hr) and urine (10-35%). Levels were detectable in plasma, skeletal muscle, liver, kidney, and skin, but near background by 3 hrs. Most radioactivity was present in feces at 24 hrs, indicating extensive biliary excretion.

Low RBC partitioning was detected, but soluble RBC COMT inhibition was noted, suggesting potent inhibitory effects of entacapone.

D.2.b. Protein Binding and Displacement

The binding of entacapone (2000 to 200000 ng/ml) or ^{14}C-entacapone (400 to 2000 ng/ml) to plasma proteins in humans and several animal species was determined after ultrafiltration.

Binding was high in all test species with some slight interspecies variations:

Species	% Unbound
human	2.0
mouse	5.3
rat	2.3
dog	10.7
rabbit	2.6
cyno monkey	2.4
pig	5.3

Human albumin strongly bound entacapone, and likely accounts for the greatest fraction of binding.

Binding of the Z-isomer to human protein was slightly higher (3.2%).

High concentrations of diazepam and ibuprofen (15 - 30μM) displaced entacapone binding from HSA (binding site II). Entacapone did not displace, nor was it displaced by other highly bound drugs (warfarin, salicylic acid, phenylbutazone, carbidopa).

D.3. Metabolism

D.3.a. Rat Plasma

Samples from the single dose rat PK study, in which animals (n = 7-8) were treated intravenously with 5 mg/kg entacapone, were analyzed for entacapone and major metabolites by

In addition to entacapone and its Z-isomer, two peaks were identified. The analytical data were consistent with their identification as the N-monodeethylated and N,N-dideethylated metabolites. The relative amounts (%) of these species over time suggested a two-step dealkylation process. Levels of the Z-isomer were relatively constant:

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	ENTAC	Z	mono-deethyl	di-deethyl
3 min	93.6	2.3	3.0	1.2
6 "	90.5	2.3	5.0	2.2
10 "	88.2	2.5	6.1	3.2
20 "	82.9	2.7	8.7	5.7
30 "	79.8	2.7	10.1	7.4
60 "	70.5	2.9	13.2	13.4
120 "	58.2	3.5	12.8	25.5

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D.3.b. Rat Urine and Bile

The main metabolites of entacapone were identified in urine of male rats (Han:Wistar, n = 11) treated orally with 30 mg/kg entacapone. Bile and urine samples were collected in another separate study from bile duct-cannulated rats treated intravenously with 3 mg/kg [^{14}C]-entacapone (n = 4M, 4F), or orally with either 10 mg/kg [^{14}C]-entacapone (n = 4M, 5F) or 90 mg/kg [^{14}C]-entacapone (n = 4M, 4F). Metabolites were extracted from urine and bile before and after hydrolysis with glucuronidases and sulfatases, isolated and purified using and extraction. Urinary metabolites in the first study were identified by Biliary and urinary metabolites in the second study were identified based on Relative amounts of the various metabolites were quantitated by

The relative amounts of entacapone and its urinary metabolites in a pooled 0-6 hr urine sample from the first study are in the following table. Entacapone and its glucuronide conjugate were the largest fractions. Various sulfate or glucuronide conjugates constituted the greatest fraction of the remaining peaks, and unconjugated metabolites were relatively minor components:

Metabolite	%
Entacapone (E)	15.5
E-glucuronide	50.4
monodeethyl-E-sulfate	8.5
E-sulfate	6.0
Z-glucuronide or sulfate	5.5
monodeethyl-E-glucuronide	4.1
Z-glucuronide	4.0
monodeethyl-E	2.3
unknown sulfate	1.8
Z isomer	0.8
O-methyl-E	0.8
unknown sulfate	0.4
N-acetyl-E	0.2

A separate sample was subjected to treatment with glucuronidase and sulfatase to further assess Phase I reactions. The relative amounts of Phase I products in a hydrolyzed samples were:

Metabolite	%
Entacapone (E)	62.3
E-monodeethyl	18.4
Z isomer	8.5
E-dideethyl	4.4
diastereomer of dideethyl	3.2
unknown	0.7
O-methyl-E	0.6
unknown	0.4
N-acetyl-E	0.4
3,4-diOH-5-nitrobenzaldehyde	0.1

These data are generally consistent with the rat plasma study in that the main phase I metabolic pathways were consecutive deethylations of the diethylamide group and isomerization to the Z-isomer. However, most urinary metabolites are sulfate or glucuronide conjugates.

Similar metabolite profiles were observed in both urinary and biliary samples from the second study. The major metabolites were glucuronides and sulfates of entacapone, its (Z)-isomer, and its N-dealkylation products. Small differences in the relative amounts of metabolites excreted in urine in intact rats and rats from which the bile was collected indicate that reabsorption of entacapone and its metabolites excreted in the bile may occur.

In summary, the main metabolites of entacapone in rat urine are conjugates of the unchanged drug, its (Z)-isomer and N-dealkylation products. Other identified metabolites resulted from reduction or cleavage of the side chain double bond, 0-methylation and reduction of the nitro group followed by acetylation.

D.3.c. Dog urine

The main metabolites of entacapone were identified in urine of two beagle dogs that were treated with a daily dose of 80 mg/kg during the subchronic toxicity study. A 0-6 hr urine sample was collected during study week 3. Metabolites were extracted from urine both as intact metabolites and after glucuronidase treatment. Quantitative estimation of the relative amounts of metabolites was performed by [redacted] Compounds not present in control urine were isolated and purified using [redacted]

Approximate percentages of the most prevalent identified metabolites in the individual dogs were:

Metabolite	%
Z-glucuronide	41, 27
E-glucuronide	32, 24
Entacapone (E)	6, 2
Z	4, 3
Z-sulfate	2, 2

Other metabolites, representing ≤ 4% of total peak area, were not conclusively identified, but spectral data suggested additional Phase I reaction products from reduction of the side chain double bond, amide hydrolysis, and nitrile hydrolysis. These metabolites were also excreted in urine as conjugates.

The main Phase II reaction was glucuronide conjugation. Sulfate conjugation was minimal.

The urine of one dog contained a high level of material (28%) corresponding to the glucuronide of 3,4-dihydroxy-5-nitrobenzaldehyde. This may have resulted from degradation of metabolites in the urine sample, since only a small amount (<1%) of nitrobenzaldeyde metabolites were present in the other dog urine sample.

In summary, the main metabolites of entacapone in dog urine were conjugates of the unchanged drug and its (Z)-isomer.

D.3.d. Comparison of metabolite profiles of experimental animals and man

Metabolite profiles of entacapone in plasma and urine samples from dogs, monkeys, rabbits, rats, and mice were determined after an oral dose of ^{14}C-labeled entacapone. Urine samples were collected during a 12 h period (exception: 24 h for one rabbit), and blood samples were drawn after 30 min and 2 h (exception: 1 h rat samples).

The profiles were compared with that of pooled human samples collected from 4 patients 30 min after a 200 mg dose of unlabelled drug.

Pooled urine samples from usually 1-4 animals (except mice, n = 12 M, 12 F) were analyzed as such to determine the percentage of unchanged drug and its (Z)-isomer. Samples of the urine pools were treated with glucuronidase/sulfatase. Identification was based on comparison of HPLC retention times and UV-spectra of previously identified metabolites. Three metabolites were further isolated from hydrolyzed urine for structural characterization. The relative amounts of metabolites were estimated from the radiochemical chromatograms.

The relative amounts of metabolites in plasma and urine samples are shown in the following tables. The percent of radioactivity excreted in urine of animals were: dog (10-15%), monkey (4-40%), rabbit (40%), rat (23%), mouse (12-15%).

Plasma Metabolites (%)

		E	E-gluc	Z	Z-gluc	3-OMe-E	Other
human	0.5 hr	55	30	4	11	-	-
dog (m & f)	0.5 hr 2 hr	64 48	4 10	9 12	5 22	5 <3	12 5
monkey (m)	0.5 hr 2 hr	51 27	38 50	- -	10 19	- -	1 4
rabbit (f)	0.5 hr 2 hr	10 <3	72 69	- -	4 4	5 18	9 7
rat (m)	1hr	24	43	-	5	19	9
mouse (m & f)	0.5 hr 2 hr	15 -	38 -	- -	- -	44 94	3 6

Urine Metabolites (%)

		E	E-gluc	Z	Z-gluc	3-OMe-E	Other
human	0-12 hr	3	80	< 3	17	-	-
dog (m & f)	0-12 hr	≤ 3	7	< 3	14	-	73
monkey (m)	0-12 hr	< 3	22	< 3	12	-	66
rabbit (f)	6 hr	< 3	12	< 3	4	-	84
rat (m)	0-12 hr	7	30	< 3	< 3	< 3	63
mouse (m & f)	0-12 hr	12	50	< 3	-	-	38

Hydrolysis of urine samples with glucuronidase or sulfatase confirmed that the major fraction of urinary metabolites are glucuronide or sulfate conjugates of entacapone or the Z isomer. Two additional metabolites were identified in hydrolysed samples from some species (but not man) that were not detected in nonhydrolyzed samples. These were:

- side chain reduction product [all species except human and mouse at 3-15%]
- nitro reduced and acetylated [monkey, 8-32%; rabbit, 6-8%]
- nitro reduced and acetylated; side chain reduced [monkey, 7%; rabbit, 12%]
- N-monodeethylated [rat, 10%]
- 3-O-methylated [rat, <3%]

To summarize the major points of entacapone metabolism:

1. The primary route of metabolism is conjugation, particularly glucuronidation, in all species.

2. Only small amounts of unchanged entacapone are excreted in urine.

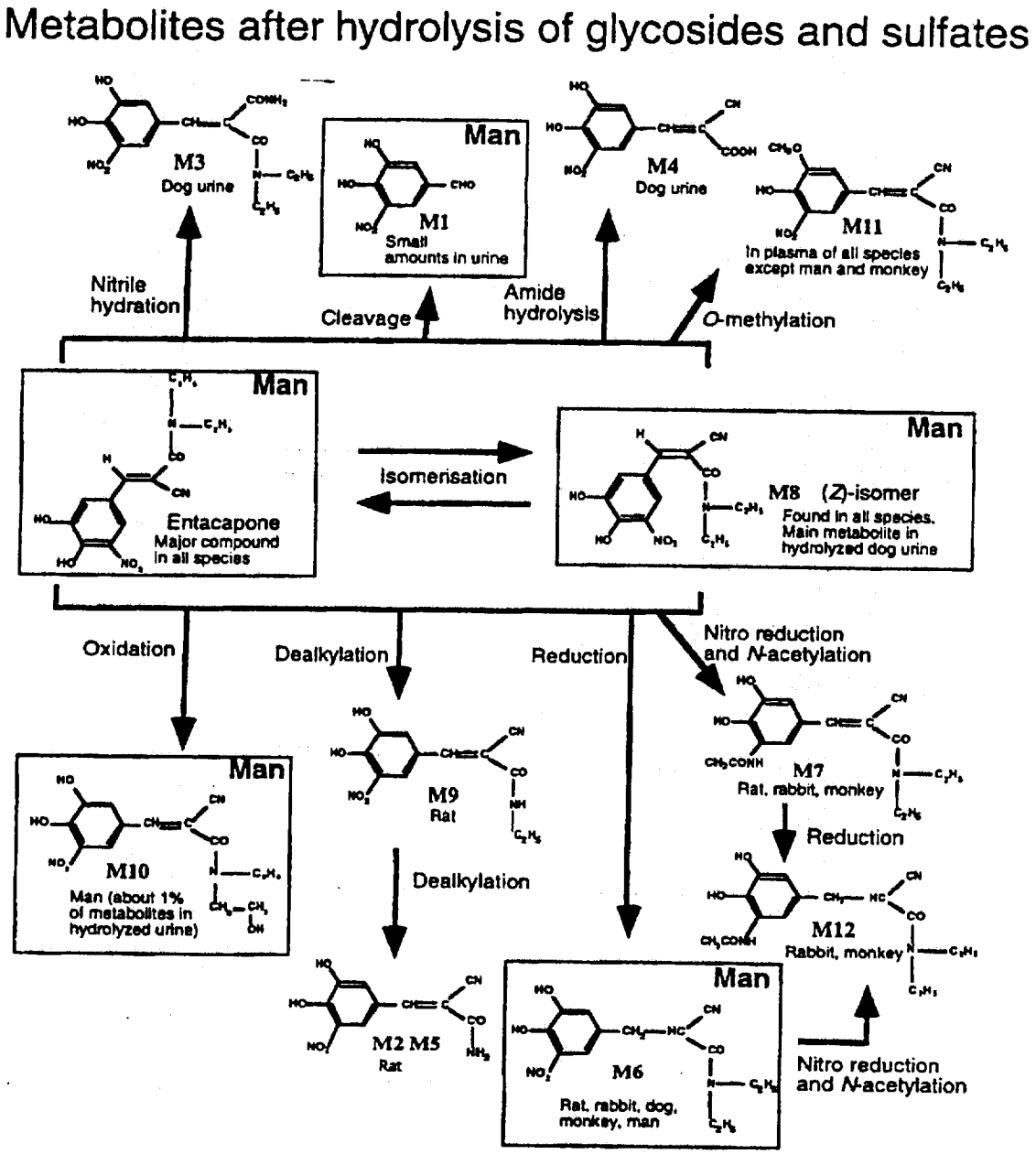
3. Isomerization to the Z-isomer is significant in humans, dog, and monkey.

4. 3-0-Methylation is significant in rat, mouse, and rabbit, but not humans.

5. Nitro reduction and subsequent acetylation of the amino group was one of the major metabolic pathways in rabbits and monkeys, but has not been observed in man.

6. Only one metabolite (alcohol oxidation product of one aminoalkyl side chain) appears to be specific to humans, but is present at <1% in urine.

The following diagram (from Sponsor Summary) summarizes entacapone Phase I metabolism among species. The nitrobenzaldehyde is shown as specific to man, but has been observed in rat and dog urine, possibly as a degradation product.



Metabolic pathways of entacapone in man and in animals based on plasma and urinary metabolites after hydrolysis of glycosides and sulfates. The metabolites in boxes have been found in human samples.

D.4. Excretion

D.4.a. Rat urinary and fecal excretion

Excretion of total radioactivity in rat (Crl:CD; n = 4/sex/treatment) urine and feces was determined after a single oral (10 or 90 mg/kg) or intravenous (3 mg/kg) dose of ^{14}C-labeled entacapone (54 µCi/mg; 1 mg/kg ^{14}C-entacapone).

Total radioactivity found in urine and feces was determined by [redacted] The relative amounts of unchanged entacapone, its (Z)-isomer and metabolites in urine were determined with radiochemically detected [redacted] Results are summarized in the following table:

Excretion of total radioactivity (% Dose)

		0-24 hr		0-7 days	
		Urine	Feces	Urine	Feces
oral	10 mg/kg	29	59	30	62
	90 "	25	58	25	69
i.v.	3 mg/kg	38	50	38	54

The feces was the major route of excretion of radioactivity from the rat. Elimination of ^{14}C-labeled entacapone was rapid as most was excreted within the first 24 hours.

Entacapone was excreted into rat urine mainly as glucuronides and sulfates of entacapone and its metabolites. After hydrolysis of the conjugates, entacapone and its (Z)-isomer represented 5 to 15 % of the radioactive dose. Less than 1.5 % of the dose was recovered in urine as free entacapone and less than 1 % as its (Z)-isomer. The excretion of unchanged entacapone into urine was estimated and discussed previously (sec. D.3.b)

D.4.b. Rat biliary excretion

Biliary excretion of entacapone was investigated in bile duct-cannulated rats (Crl:CD; n= 4-5/sex/treatment) following single oral (10 or 90 mg/kg p.o.) and intravenous (3 mg/kg) doses of ^{14}C-labeled entacapone (S.A. = 54 µCi/mg; 1 mg/kg ^{14}C-entacapone).

Enterohepatic circulation was investigated in conscious rats linked in pairs by bile duct-duodenum cannulae. Bile from a donor rat that received ^{14}C-entacapone was infused into duodenum of a recipient rat (4 pairs of males per treatment).

Total radioactivity found in bile, urine and feces of the bile duct cannulated rats was determined by [redacted] The relative amounts of unchanged entacapone, its (Z)-isomer and metabolites in urine were determined with radiochemically detected [redacted] Results are summarized in the following table:

Excretion of total radioactivity (% Dose)

		Bile	Urine	Feces
oral	10 mg/kg	26.7	26.2	25.1
	90 "	34.4	19.5	17.9
i.v.	3 mg/kg	51.3	36.6	3.3

Biliary excretion of total radioactivity following an i.v. dose was extensive, and most occurred within the first hour. After oral dosing, about 15-25% of total radioactivity was excreted into bile within the first 6 hours.

Results on the metabolic profile of entacapone in bile were discussed in D.3.b. Free unchanged entacapone represented about 10% of the radioactivity found in bile (1-5% of the radioactive dose). The main metabolites of entacapone in rat bile were glucuronides and sulfates conjugates of entacapone, the Z-isomer, and deethylation products.

The study in paired bile duct-duodenum cannulated rats estimated the enterohepatic circulation of entacapone as approximately 9 %.

D.4.c. Rat Milk Excretion

Excretion of total radioactivity into milk of lactating female rats (Crl:CD; n = 4; 1-2 rats sampled per timepoint) was investigated following a single oral 10 mg/kg dose of [^{14}C]-labeled entacapone (S.A. = 54 µCi/mg; 5 mg/kg ^{14}C-entacapone, 75 µCi/rat). Total radioactivity in plasma and milk was determined by

The radioactivities found in rat milk samples and the ratio of the milk:maternal plasma radioactivity are summarized in the following table:

	Rat #	total RA* (ng eq/g milk)	milk: plasma
1 hr	2	7030	0.94
	4	1740	0.33
6 hr	3	2320	2.6
24 hr	3	61	43
	4	880	25

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Based on these very limited data, entacapone and/or its metabolites are excreted in rat milk.

D.4.d. Dog Urinary and Fecal Excretion

The amount of total radioactivity excreted in beagle dogs (n = 2M, 2F) was determined after a single dose administration of either 20 mg/kg p.o. (capsule) or 5 mg/kg i.v. ^{14}C-labeled entacapone (0.5 mg/kg ^{14}C-entacapone). Excretion of unchanged entacapone into urine was also estimated.

Total radioactivity found in urine and feces was determined by [redacted] The relative amounts of unchanged entacapone, its Z-isomer and metabolites in urine were determined by [redacted] with radiochemical detection. Results are summarized in the following table:

Excretion of total radioactivity (% Dose)

		0-24 hr		0-7 days	
		Urine	Feces	Urine	Feces
oral	20 mg/kg	16	41	17	55
i.v.	5 mg/kg	18	51	19	65

These data indicate an incomplete recovery of total radioactivity in excreta. The sponsor attributes loss to excretion while animals were removed from the cage, during blood sampling, or other nonspecific means.

Free entacapone and the Z-isomer were 0.5 % or less of the total radioactive oral dose in dog urine, as the drug was mainly excreted as glucuronide and sulfate conjugates of the parent, the Z-isomer and other metabolites (see interspecies comparison data in section D.3.)

SUMMARY AND EVALUATION

Entacapone (ENT) is a new chemical entity developed for use as an adjunct to levodopa preparations for treatment of Parkinson's disease. The therapeutic effect of ENT is purportedly attributed to inhibition of the enzyme catechol-O-methyl transferase (COMT), which catalyzes the methylation of catechols at the 3-hydroxyl position. By inhibiting the 3-O-methylation of L-DOPA, a primary route of L-DOPA inactivation, ENT increases plasma exposures to L-DOPA and thereby improves its pharmacokinetic profile and antiparkinsonian activity. ENT is the second drug candidate of this class; tolcapone (TOL; TASMAR™) was approved for the same indication in January, 1998.

The COMT-inhibitory activity of ENT was demonstrated in several *in vitro*, *in vivo* and *ex vivo* experiments. ENT was very similar to TOL in most assays, including enzyme specificity and *in vivo* potency. One potential difference in the pharmacological activity of the two compounds is their relative degrees of central versus peripheral COMT inhibition. For instance, ENT increases and TOL decreases rat striatal homovanillic acid (HVA) levels, the 3-O-methylated DA metabolite generated by COMT activity after oxidation of DA to DOPAC. However, evidence from *ex vivo* and microdialysis studies suggest that central COMT inhibition does occur with high doses of ENT. Distribution studies suggest that both ENT and TOL poorly penetrate the BBB. Thus, the relative contribution of central and peripheral COMT inhibition to the therapeutic activity of either compound has not been clearly established, but in view of the relatively high doses in animals required to elicit central effects, it is likely that peripheral COMT inhibition is the major contributor. Both compounds effectively increased plasma and brain L-DOPA levels, and were active in animal models of PD in an oral dose range of 10-30 mg/kg.

The level of toxicological testing of ENT submitted in the NDA meets the general requirements of that typically expected for a drug developed for a chronic use indication. However, significant flaws were indentified in the design, conduct, and outcome of some of the major studies (carcinogenicity, reproductive toxicology, chronic toxicology) that impact the application's approvability, product labeling, and/or Phase IV commitments.

A major issue was raised in each of the carcinogenicity studies. Each issue requires an individual assessment, but the outcome of these assessments must be coupled to generate an overall conclusion regarding the adequacy of the testing, and its regulatory implications.

The issue in the mouse study was the occurrence of significantly increased mortality in the high dose group of both sexes. The effect was most evident in females, as mortality in HDF was approximately 40% by the end of month 4, and exceeded 50% by the end of one year; only 9 of 50 HDF remained when the group was terminated at week 95. Survival of HDM at termination (week 104) was 28%. As indicated in the review of Roswitha Kelly, the Agency statistician (see Appendix 1), survival in this dose level does not appear to be sufficient to adequately assess the carcinogenic potential (i.e., late-developing tumors) of ENT. With the "failure" of this group, the suitability of the MD (100 mg/kg) for risk assessment purposes was considered, but there was no evidence from any other mouse toxicology studies that this dose was within a reasonable range of an MTD. In fact, no toxicities or indication of drug-induced lethality were evident in the 13-week study of doses up to 400 mg/kg (600 mg/kg was the high dose from week 9-13). Carcinogenicity study dose selection was based on saturation of drug absorption in the 13-week study, but only parent drug was measured. Since ENT is rapidly and extensively metabolized, the toxicokinetic analyses to demonstrate saturation of absorption should have included the major drug-

related species. Also, MDM surviving to terminal sacrifice were not evaluated histopathologically. Based on the absence of animal exposures to appropriate (near MTD) levels of ENT for a sufficient duration and the inadequate histopathological assessments, the study is not considered a valid assessment of the carcinogenic potential of the drug. Moreover, the estimated plasma mouse exposures at the HD exceeded the estimated human exposures at the maximum recommended daily dose by only 5-7 times.

In the rat carcinogenicity study, renal tubular benign adenomas and malignant carcinomas were found in 6 HDM and 5 HDM, respectively (HD = 400 mg/kg). This dose level was associated with plasma exposures that exceeded estimated human exposures at the maximum recommended daily dose by 8-25 times. The sponsor proposes that these tumors are due to altered renal handling and deposition of a male rat-specific protein, α2-microglobulin (α2-μG). This proposed mechanism is established in the literature, and not considered relevant to humans. The sponsor has presented some data to support their hypothesis, including histological and immunocytochemical evidence of the presence of hyaline protein droplets and α2-μG in male rat kidney sections from other toxicology studies (preliminary Seg I/III study, 6 week treatment; 52-week rat study). However, as indicated by the contract pathologist that conducted the immunocytochemical study, several other pathological changes that are usually associated with altered α2-μG handling were absent from the sections, raising doubts on the involvement of this mechanism. Additional factors that cast doubt on the exclusive involvement of α2-μG deposition in ENT-induced renal tumorigenesis include the occurrence of a low number of rarely spontaneous renal tubular carcinomas in female rats, the renal tumorigenic potential of TOL, and the positive *in vitro* clastogenicity findings with ENT.

A final factor to be considered in weighing the evidence regarding the species (i.e., male rat) specificity and relative human carcinogenic risk of ENT relates back to the validity of the mouse study. Obviously, one reason for assessing two species in CA bioassays is to address the question of trans-species carcinogenicity. Thus, the identification of the tumor signal in the rat study increases the need for a valid mouse study, and precludes any suggestions or conclusions regarding the potential species-specific tumorigenic effects of ENT.

The reproductive toxicology program was deficient from a toxicological standpoint in that the Segment I and III studies were not conducted with sufficiently high doses; the high dose of 700 mg/kg/day was not maternally toxic. Neither study revealed clearly treatment-related effects on fetal morphology, development or pup maturation, although the incidence of fetal eye anomalies slightly exceeded historical control levels in the Segment I study. Maternotoxicity was also not evident at the HD of 1000 mg/kg in the rat Segment II study. A TK analysis in a preliminary study indicated that top dose selection in that study may be acceptable based on the finding of saturation of ENT absorption and/or elimination in the dose range of 350-500 mg/kg, but only levels of the parent compound were measured. Fetal assessments in the rat teratology study revealed some delays in ossification and increased incidences of enlarged fronto-nasal sutures and wavy ribs at the HD, which are noted as occurring below maternotoxic dose levels. Maternal rat exposures at the high doses used in the rat reproduction studies (700-1000 mg/kg/day) exceeded estimated human exposures at the maximum recommended daily dose by approximately 25-30 times.

The rabbit Segment II study was valid as maternotoxicity was achieved (suppression of weight gain at MD = 100 mg/kg/day; weight loss at HD = 150 mg/kg/day). Potential abortifacient properties of ENT were evident at these doses; TOL also induced abortions in rabbits. The incidences of late and total

resorptions and percentage of post-implantation losses appeared increased relative to concurrent and historical controls in MD and HD ENT-treatment groups, but the differences were not statistically significant. Fetal assessments suggested developmental effects at the HD (incomplete ossification, higher incidence of small fetuses). Maternal exposures in rabbits at the HD were in the range of those expected to be achieved in humans receiving the maximum recommended dose.

The teratogenicity of ENT in combination with levodopa was not assessed. Since ENT is always given in combination with levodopa, which has been associated skeletal and visceral malformations in rabbits, the Pregnancy categorization and labeling for ENT should reflect the effects associated with levodopa, and recognize the possibility that ENT may potentiate the effects based on its pharmacological mechanism of action. Potentiation of fetal malformations by Sinemet was noted in Segment II combination studies of TOL and levodopa in rabbits.

Despite the deficiencies in reproductive toxicology testing of ENT, the value of requesting a repeat of any studies needs to be considered within the context of the proposed indication and use. Obviously, PD is a disease that mainly affects a female patient population that is approaching or beyond the age of child-bearing potential. The teratology studies, arguably the most important of the battery, were generally acceptable (support for the rat high was questionable) and revealed some important findings. Coupled with the inclusion of labeling statements on levodopa-associated fetal malformations, it is expected that this information will be sufficient to alert physicians to exercise appropriate precautions when faced with the (likely rare) prospect of prescribing ENT to women of child-bearing potential. Additional fertility testing would also probably provide little additional information, as the doses administered were relatively high (plasma exposures well in excess of anticipated human exposures), and appeared to approach a level near absorption saturation. In addition, the chronic animal toxicology studies did not provide any indication of potential reproductive toxicities of ENT.

The designs of the chronic toxicology studies were adequate to assess potential long-term drug effects, but one major issue raises a concern over the integrity of the information provided. The initial report for the rat study stated that chronic myocarditis was the only treatment-related effect, presumably due to an acceleration of a spontaneous aging process mediated by endogenous catecholamines. However, kidney sections from this study were re-evaluated apparently *after* the observation of renal tumors in the 2-year bioassay to clarify and characterize lesion development. The standard H-E staining of apparently newly cut kidney sections revealed an increased incidence of two renal pathologies in HD males, chronic progressive nephropathy (CPN: 3/20 ConM, 11/20 HDM) and enlarged nuclei (EN: 1/20 ConM, 18/20 HDM). The number of HDF identified with these pathologies was also higher than in controls (CPN: 0/20 ConF, 2/20 HDF; EN: 8/20 ConF, 14/20 HDF). Strangely, the initial histopathological study had identified only 5 HDM, but 6 HDF with CPN. It is noted that the second analysis in which the treatment effect in HDM was noted was not conducted by a blinded observer, which raises the troubling concern of bias. The discrepancy between the two studies, and the determination of which results are correct, is significant in light of the sponsor's proposal that renal pathologies are a male rat-specific phenomenon. While additional staining techniques (trichrome-stain for hyaline protein droplets, immunocytochemical analyses for α2-μG) were used to identify some of the characteristic features of α2-μG nephropathy, other pathological changes typically associated with α2-μG deposition were not found (necrosis, epithelial cell proliferation, granular casts, linear mineralization of the renal papilla, urothelial hyperplasia). Finally, two HDF dogs in the one-year study were diagnosed with renal tubular vacuolation and degeneration. The finding was not considered treatment-related by the sponsor, and the description was not sufficient to

determine the extent of similarity to the rat lesion. In view of the rat findings, a potential treatment-relationship should be considered. Coupled with the aforementioned concerns, the finding raises further doubts on the species-specificity of ENT-associated renal pathologies.

An accurate and descriptive characterization of the rat renal pathologies associated with ENT is also necessary to determine their relative similarities to those of TOL. The sponsor has not clearly defined chronic progressive nephropathy, but the following Table from a review by Hard et al. (Environ Health Perspectives, 99:313, 1993) suggests some similarities between CPN and the renal "tubulopathy" described for rats treated with TOL (tubulopathy = a summarizing term used by the pathologist when either tubular cell degeneration, tubular single cell necrosis, tubular cell hyperplasia, and/or karyocytomegaly in the straight portion of proximal tubules):

Table 4. Summary of the histopathology of spontaneous chronic progressive nephropathy of aging rats.

Thickening of tubular and glomerular basement membranes
Basophilic segments of proximal convoluted tubules with sporadic mitoses indicative of tubule cell proliferation
Tubular hyaline casts of proteinaceous material originating in the more distal portion of the nephron, mainly in the medulla, and later plugging a considerable length of the tubule
Focal interstitial aggregations of mononuclear inflammatory cells within areas of affected tubules
Glomerular hyalinization and sclerosis
Interstitial fibrosis and scarring
Tubular atrophy involving segments of proximal tubule
Chronically in advanced cases, occasional hyperplastic foci in affected tubules
In some advanced cases, accumulation of protein droplets in sporadic proximal tubules

While some of the pathological findings with the two compounds may have been similar, the incidence and possibly severity of the lesions associated with TOL was greater than that seen with ENT. Also, there was no suggestion of a possible gender difference in rats treated with TOL. The degree or severity of renal pathologies associated with TOL increased with treatment duration (i.e., 2-year vs. 1-year study), but the incidence of CPN in the 2-year ENT bioassay was similar across dose groups (34-43 males/group affected; 10-19 females/group affected). The latter observation is considered unusual, and raises additional questions on the description and/or diagnoses of the renal pathologies associated with ENT. Without this information, an the need for, or contents of, an accurate labeling statement regarding the renal toxicity of ENT in rats, similar to that which appears in TASMAR labeling, cannot be determined.

A consistent finding in the rat and dog chronic toxicology studies was a small, but statistically significant reductions in red cell parameters (count, Hb, Hct, MCV). The profile of changes (hypochromic, microcytic anemia) is consistent with an iron deficiency due to chelation by the catechol structure. A special study revealed the iron-chelating properties of entacapone at high *in vitro* concentrations.

Toxicokinetic data from the critical toxicology studies and their relationship to estimated human exposures at the maximum recommended daily dose (MRDD = 1600 mg) are summarized in a following

Table. Comprehensive toxicokinetic analyses were not conducted in many studies; rather, plasma levels were determined at one or a few time points. Those data were considered sufficient only for demonstrating absorption, and are not included in the table. Also, the human exposure value used in the calculation was derived by multiplying the AUC associated with a single dose (1.5 µg.hr/ml at 200 mg) by the recommended maximum number of doses per day (8). This method was considered valid by the Biopharmaceutics reviewer (Dr. Syed Al-Habet) because of ENT's short half-life and linear kinetics, and was necessary because the sponsor did not provide substantial PK information from patients receiving the maximum daily dose.

Comparative Entacapone Exposures Among Species

Species	Duration (time of measure)	Dose		AUC (µg.hr/ml)	AUC ratio to MRDD
Human	-	1600 mg (MRDD)		12	-
Dog	52 wk (wk 50)	20	M	3.8	0.3
		*	F	4.2	0.3
		80 *	M	20	1.7
			F	22	1.8
		300	M	142	11.8
			F	153	12.8
Rat	CA Study (avg of wks 26, 52, 78, 104)	20 *	M	24	2.0
		*	F	34	2.8
		90	M	63	5.2
			F	65	5.4
		400	M	230	15.3
			F	282	19.2
Mouse	13 wk	20	M	1.4	0.1
			F	2.6	0.2
		200	M	30	2.5
			F	72	6.0
		600[a]	M	83	6.9
			F	111	9.2
Preg Rat	Developmental Toxicity (d. 15)	40		12[b]	0.5
		160 *(F_1)		120[b]	5.0
		1000 *(F_0)		820[b]	68.3
Preg Rabbit	Developmental Toxicity (d. 19)	40 * (F_0, F_1)		2.8[b]	0.1
		100		6.4[b]	0.3
		300		18.6[b]	1.6

APPEARS THIS WAY ON ORIGINAL

* NOAEL; **MRDD** = 200 mg, 8 times daily (AUC ~ 1.5 µg.hr/ml x 8);

[a] HD in 2-year mouse carcinogenicity study

[b] AUC_{0-10} multiplied by two (# doses per day)

The genetic toxicology battery of testing was complete and generally acceptable. Entacapone was positive in *in vitro* clastogenicity tests (small colony formation in mouse lymphoma, chromatid breaks in human lymphocytes), furthering the possibility that renal tumors may not be solely due to α2-μG. The *in vivo* micronucleus test was negative (ENT alone and in combination with Sinemet). The suitability of this model was not clearly established (no evidence of bone marrow toxicity or distribution to bone after a single dose), but the test doses were considered appropriate. ENT was also negative in the Ames test, alone and in combination with Sinemet, and an *in vivo* rat liver DNA repair test. ENT did not bind DNA.

The possibility that the toxicity of either ENT or Sinemet is enhanced when the drugs are administered in combination was assessed in 13-week studies in rats and monkeys. Neither study revealed any unexpected toxicological interactions. However, in rat combination studies of ENT and benserazide (a decarboxylase inhibitor available in Europe), enlarged nuclei (minimal karyomegaly) in cells of the proximal convoluted tubules were observed in male and female rats treated with the combination and with ENT alone. The findings appear similar to those described in toxicology studies of TOL. This transgender observation raises the possibility that renal pathologies not associated with α2-μG deposition can result from ENT administration.

Some interesting toxicokinetic interactions were observed in the 13-week studies. As expected, ENT increased L-DOPA exposures in rats, but reduced carbidopa exposures, possibly due to interference with carbidopa absorption. In monkeys, peak plasma L-DOPA levels were reduced by ENT, and total exposures to L-DOPA were not increased by ENT, raising the possibility that ENT decreased or delayed the absorption of L-DOPA and/or carbidopa at high doses.

Safety pharmacology studies did not identify any major organ system side effect concerns, but the adequacy of some of testing conditions may be questioned. For example, the effects of ENT alone and in combination with intravenous monoamines (tyramine, EPI, NE, ISO) were evaluated in a hemodynamic study in anesthetized rats. Effects were negligible, but ENT was administered approximately 90 min before the monoamines; thus, most drug would have been eliminated by the time of monoamine administration. In fact, the lack of an ENT effect is probably the strongest indication of a study design failure since ENT inhibits an inactivation mechanism of catecholamines, and should enhance their cardiovascular effects. However, the significance of concerns with this test battery at this stage development is rather minimal, since the safety concerns should have been evaluated in detail in clinical studies.

One interesting series of experiments that were addressed in the safety pharmacology section were studies on the effects of entacapone on oxidative phosphorylation, a primary biochemical mechanism of energy production. These studies were undertaken based on the chemical similarity of ENT's nitrocatechol moiety and the known uncoupler 2,4-dinitrophenol (2,4-DNP). TOL was also included in the comparative studies, which included biochemical studies of mitochondrial respiration, a functional assessment at the tissue level (isolated guinea pig heart), and an *in vivo* study of hyperthermia in mice since heat dissipation is a consequence of oxidative phosphorylation uncoupling. Interestingly, TOL appeared to be the most potent inhibitor of ox-phos in the mitochondrial preparation (5 times more potent than DNP; 20 times more potent than ENT). In the isolated heart, TOL was 10 times more potent than ENT in reducing myocardial ATP content. In the *in vivo* study, the hyperthermic effects of TOL and DNP were small, and of questionable toxicological significance; ENT caused a slight hypothermia. In view of the diverse toxicities that may be a consequence of uncoupling of oxidative phosphorylation, these studies may define

an important difference in the safety of the two, otherwise similar, compounds. However, the proposed maximum human daily dosage of ENT (2000 mg) is notably higher than that of TOL (600 mg). Therefore, the relative difference in human risk associated with ENT and TOL therapy may not be as great as that suggested by this battery of studies.

The pharmacokinetic/ADME studies suggested generally similar properties among species. Bioavailability was low to moderate. The elimination half-life is short; hence, accumulation did not occur in animals after repeated dosing. The volume of distribution was small, and plasma protein binding was extensive (>90%). ENT is metabolized mainly by conjugation (primarily glucuronidation). In humans and dogs, significant isomerization to the active Z-isomer was noted; this metabolite is also mainly excreted by conjugation. One minor (<1%) metabolite (amino side chain alcohol oxidation product) was unique to humans. ENT-related material is excreted mainly in feces (approximately two-thirds). Only a small fraction of drug is excreted unchanged in the urine.

In summary, a major aspect of the animal toxicology testing of ENT, the carcinogenicity bioassays, will require a significant regulatory action. The mouse study is not considered valid because a low survival rate in high dose animals resulted in an insufficient exposure duration to suitably high (i.e., near MTD) levels of drug. The mid-dose of the study was not demonstrated as sufficiently close to an MTD, and males of this group were not evaluated histopathologically. These deficiencies should be addressed before the study can be considered acceptable. Alternatively, a short-term alternative mouse carcinogenicity study model could be explored. In rats, the positive carcinogenicity signal (renal tumors) was suggested as a male rat-specific event (α2-μG deposition), but the supporting evidence was not convincing to support an exclusive role for α2-μG in rat renal tumorigenesis. The adequacy of some reproductive toxicology studies was not conclusively established, but additional studies are not warranted.