CENTER FOR DRUG EVALUATION AND RESEARCH

APPLICATION NUMBER:

21-485

PHARMACOLOGY REVIEW

REVIEW AND EVALUATION OF PHARMACOLOGY/TOXICOLOGY DATA:

Reviewer Name Paul Roney
Division Name Division of Neuropharmacological Drug Products
HFD# 120
Review Completion Date March 5, 2003

Review number Original
IND/NDA number NDA 21-485
Serial number/date/type of submission June 24, 2002
Information to sponsor: Yes ( ) No ( )
Sponsor (or agent): Orion Pharma Inc

Drug:

Code Name: LCE, Entacapone Combi
Generic Name: Entacapone/Levodopa/Carbidopa Product
Trade Name: Stalevo

Chemical Name:

Entacapone- (E)-2-Cyano-3-(3,4-dihydroxy-5-nitrophenyl)-N,N-diethyl-2-propenamide
Levodopa- 3-Hydroxy-(-)3-(3,4-dihydroxyphenyl-L-alanine-L-tyrosine
Carbidopa- (S)-alpha-Hydrazino-3,4-dihydroxy-alpha-methyl-benzenepropanoic acid

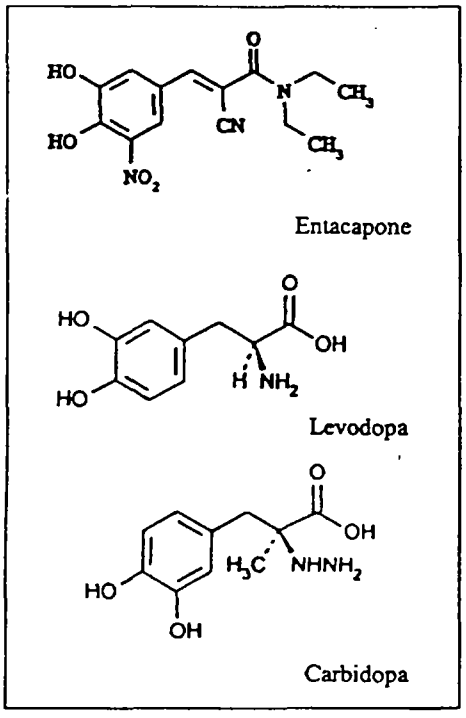
CAS Registry Number:

Entacapone 130929-57-6
Levodopa 59-92-7
Carbidopa 38821-49-7

Molecular Formula/ Molecular Weight:

Entacapone $C_{14}H_{15}N_3O_5$ / 305.3
Levodopa $C_9H_{11}NO_4$ / 197
Carbidopa $C_{10}H_{14}N_2O_4$ / 244

Structure:



Relevant INDs/NDAs:

Combination	IND 60,554
Entacapone	IND 37,771, NDA 20-796
Levodopa/carbidopa (Sinemet)	IND 7,160, IND 25,370, NDA 17-555, NDA 19-856

Drug Class:

Entacapone-	Catechol-O-Methyl Transferase (COMT) Inhibitor
Levodopa-	Dopamine Precursor, Dopamine Agonist
Carbidopa	Aromatic-L-Amino Acid Decarboxylase Inhibitor

Indication: Parkinson's Disease

Clinical formulation: Tablets with three strengths of levodopa/carbidopa

Formulation	Entacapone	Levodopa	Carbidopa
1	200 mg	50 mg	12.5 mg
2	200 mg	100 mg	25 mg
3	200 mg	150 mg	37.5 mg

Route of administration: Oral

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PHARMACOKINETICS

Toxicokinetics of Carbidopa in the Rat Following 5-Day Repeated Oral Administration
Study: PR011214
Location: Volume 1.32, Page 224
Method: 9 week old Wistar rats (8/sex) were administered 400 mg/kg carbidopa for five days. On Day 5, blood samples were withdrawn for determination of carbidopa levels according to the following schedule (b.d. = before dosing):

Group	Animal Numbers		Sampling times							
	Males	Females	b.d.	1 h	2 h	4 h	6 h	8 h	12 h	24 h
1	101-104	151-154	x		x		x		x	
	105-108	155-158		x		x		x		x

Figure 1, from page 15 of Report PR011214

	Male	Female	Combined
Cmax (ug/ml)	1.42	2.57	1.77
AUC_{0-24} (ug-hr/ml)	13.4	16.7	15.0

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TOXICOLOGY

The following section is excerpted from Dr. T.D. Steele's NDA Review of entacapone (NDA 20-796).

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

GLP Research Report #: F93061210438
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

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 (assay: ——

Figure 2, from page 22 of Dr. T.D. Steele Review of Entacapone (NDA 20-796)

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).

Figure 3, from page 23 of Dr. T.D. Steele Review of Entacapone (NDA 20-796)

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).

Figure 4, from page 24 of Dr. T.D. Steele Review of Entacapone (NDA 20-796)

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

GLP Research Report #: NRO 68/943243
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 (assay: [redacted]

Figure 5, from page 25 of Dr. T.D. Steele Review of Entacapone (NDA 20-796)

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

Figure 6, from page 26 of Dr. T.D. Steele Review of Entacapone (NDA 20-796)

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

Figure 7, from page 27 of Dr. T.D. Steele Review of Entacapone (NDA 20-796)

28-Day Oral Toxicity Study in the Rat for Qualification of Carbidopa Related Impurities

Study no: **PR011199**
Volume #, and page #: 1.32 / Page 1
Conducting laboratory and location: **Orion Pharma, Espoo, Finland**
Date of study initiation: **October 29, 2001**
GLP compliance: **Yes**
QA report: yes (X) no ( )
Drug, lot #, radiolabel, and % purity: **Pure Carbidopa (Q339/P21) 100%;**

Impure Carbidopa (Q339/P30) **(impurities in table below)**

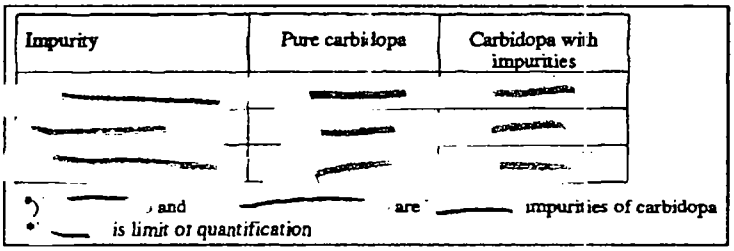


Figure 8, from page 16 of Report PR011199

Formulation/vehicle: 0.5% aqueous methyl cellulose solution

Methods (unique aspects):
Dosing:

Species/strain:	Rat, Brl:WIST Han@Mol
#/sex/group or time point (main study):	10/sex/dose
Satellite groups used for toxicokinetics:	
Age:	5 weeks
Weight:	77 ± 2 g (males); 70 ± 2 g (females)
Doses in administered units:	0, 400, 400 mg/kg
Route, form, volume, and infusion rate:	Oral gavage, 10 ml/kg

Observations and times:

Clinical signs:	1X/day
Body weights:	1X/week
Food consumption:	1X/week
Ophthalmoscopy:	Not done
EKG:	Not done
Hematology:	Week 4
Clinical chemistry:	Week 4
Urinalysis:	Week 3
Gross pathology:	Day 28-29
Organs weighed:	Day 28-29
Histopathology:	Complete
Toxicokinetics:	Not done
Other:	

Results:

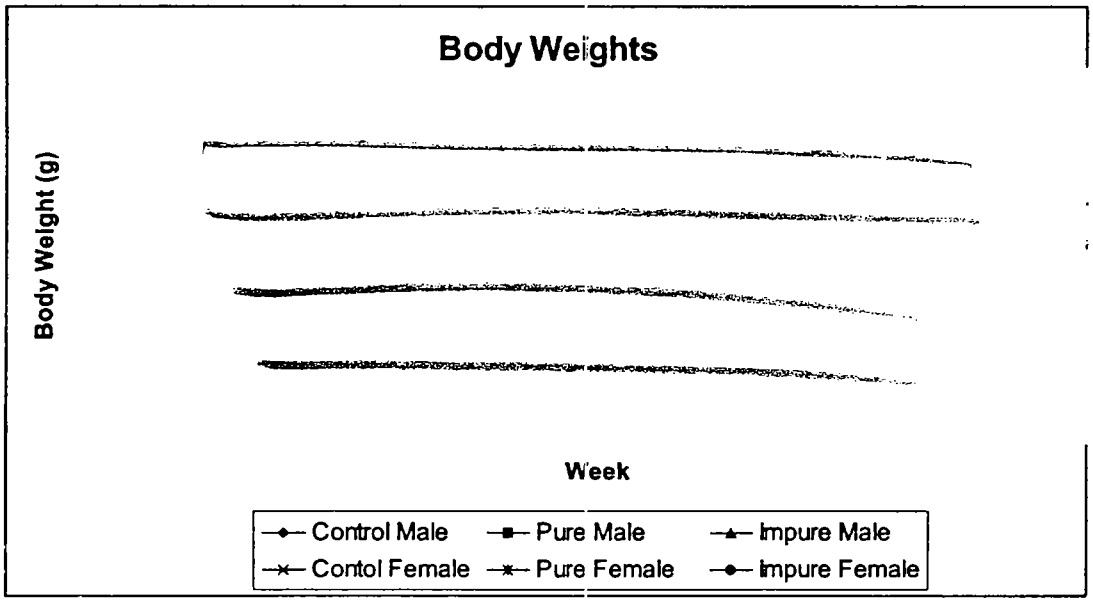
Mortality: None

Clinical signs:

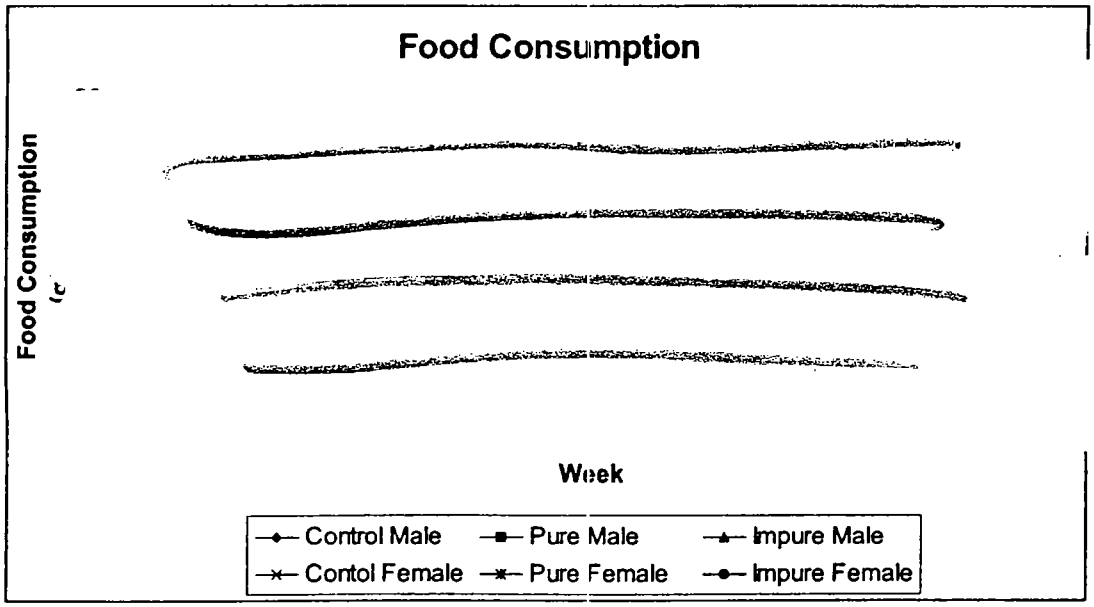
Red Staining Around nose (total observations/280 observations)

	Males	Females
0 mg/kg	6	1
400 mg/kg Pure Carbidopa	6	5
400 mg/kg Impure Carbidopa	7	3

Body weights: 5% decrease in body weight in females and 6-9% in males.



Food consumption: Somewhat decreased food consumption in treated groups



Ophthalmoscopy:	Not done
Electrocardiography:	Not done
Hematology:	No significant effects
Clinical chemistry:	No significant effects
Urinalysis:	No significant effects
Organ weights:	Increased relative liver weight in the female impure carbidopa group (3.60 versus 3.41 in controls and 3.44 in pure carbidopa).
Gross pathology:	No significant effects
Histopathology:	No significant effects
Toxicokinetics:	Not done

Key study findings:

1. Slightly decreased body weight were observed in the both carbidopa treated groups. Both carbidopa treated groups also had decreased food consumpiton.
2. Increased relative liver weight was observed in females administered impure carbidopa. In the absence of liver histopathology, the significance of this observation is uncertain.
3. No other adverse effects were noted.

REPRODUCTIVE TOXICOLOGY

Combination of Entacapone, Levodopa, and Carbidopa: Rabbit Developmental Toxicity Study

Study No: — 6831-100
Volume #, and page #: Volume 30, page 1
Site and testing facility: [redacted]
GLP compliance: Yes
QA- Reports Yes (X) No ( ):
Lot and batch numbers: 032210
Protocol reviewed by Division Yes ( ) No (X):

Methods:

- Species/strain: Rabbits, Hra:(NZW)SPF
- Doses employed:

Group	Entacapone	Levodopa	Carbidopa
1 (Control)	0	0	0
2 (Low Combination)	40	40	10
3 (Mid Combination)	80	40	10
4 (High Combination)	150	40	10
5 (Entacapone)	150	0	0
6 (Levodopa/Carbidopa)	0	40	10

- Route of Administration: Oral
- Study Design: Segment 2 study; Dosing GD 6-20; sacrifice day 29
- Number of animals/sex/dosing group: 20 females/dose
- Parameters and endpoints evaluated: fetal alterations
- Statistical evaluations: ANOVA

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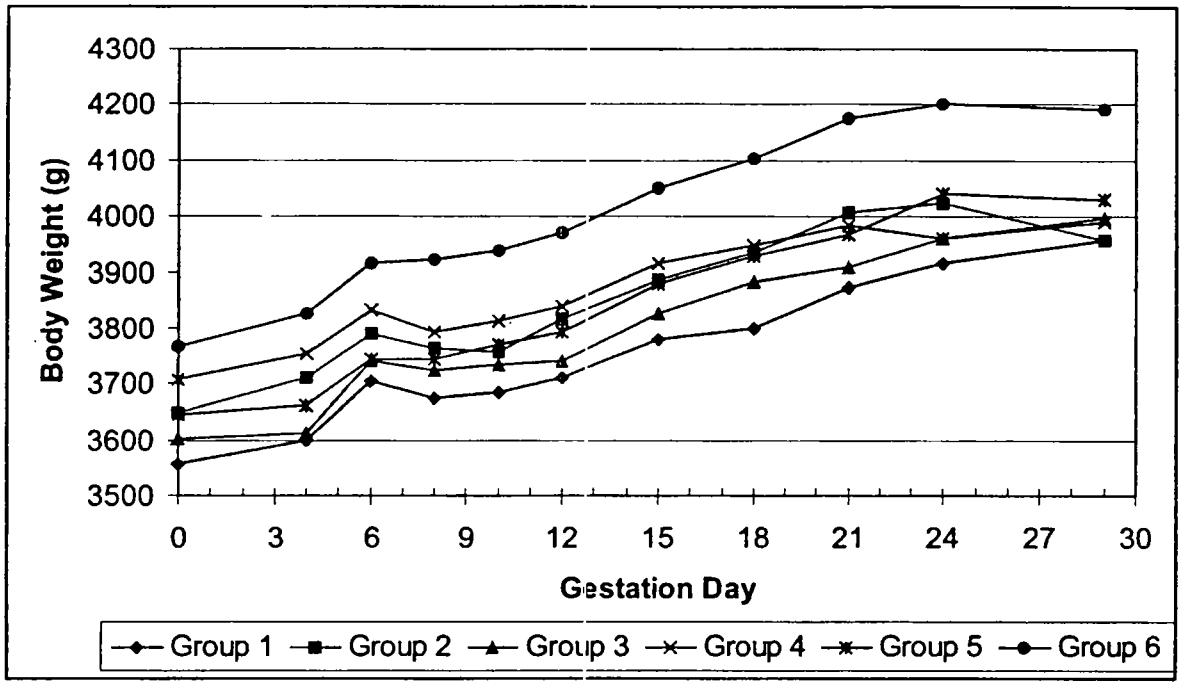
Results:

- Clinical signs: None
- Mortality: One death in groups 1, 3, and 4
- Body weight: No effect



- Food consumption: No effects
- Toxicokinetics:
- Embryo-fetal Development
  - In-life observations:
  - Terminal and Necroscopic evaluations:
  - Dams: one dam in group 4 had an abortion; no other effects

Group	1	2	3	4	5	6
Pregnant Dams	20	20	19	18	20	20
Aborted	0	0	0	1	0	0
Died	1	0	1	1	0	0
No viable fetuses	2	0	0	0	0	0
Dams with viable fetuses	17	20	18	16	20	20
Corpora Lutea/dam	9.7	10.5	9.3	10.0	9.9	9.9
Preimplantation loss(%)	1.8 (18)	0.8 (6.1)	0.7 (8.7)	1.6 (16)	1.0 (8.9)	1.7 (15)
Implantation Sites/dam	7.9	9.7	8.6	8.4	8.9	8.2
Early resorptions	0.7	0.1	0.4	0.0	0.1	0.2
Late resorptions	0.1	0.4	0.3	0.1	0.1	0.4
Dead fetuses	0.0	0.0	0.0	0.0	0.0	0.0
Postimplantation loss (%)	0.7 (12)	0.4 (4.5)	0.7 (7.8)	0.1 (1.3)	0.2 (2.1)	0.6 (7.6)
Live Fetuses/dam	7.2	9.3	7.8	8.3	8.8	7.6
Mean Fetal Weight (g)	41.92	38.61	40.47	41.33	40.87	42.17
Adjusted Fetal Weight	41.56	39.76	39.91	41.28	41.41	41.33

- Offspring: no effect on fetal weight or incidence of fetal variations or malformations.

Summary of external malformations and variations

Group	1	2	3	4	5	6
Fetuses Examined	136	185	141	132	175	152
Malformations						
Fetal Incidence(%)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
Litter Incidence (%)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
Variations						
Fetal Incidence(%)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	2 (1.3)
Litter Incidence (%)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	2 (10)
Combined						
Fetal Incidence(%)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	2 (1.3)
Litter Incidence (%)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	2 (10)

Summary of visceral malformations and variations

Group	1	2	3	4	5	6
Fetuses Examined	136	185	141	132	175	152
Malformations						
Fetal Incidence(%)	0 (0)	0 (0)	0 (0)	1 (0.8)	1 (0.6)	4 (2.6)
Litter Incidence (%)	0 (0)	0 (0)	0 (0)	1 (6.3)	1 (5.0)	2 (10)
Variations						
Fetal Incidence(%)	18 (13)	41 (22)	28 (20)	26 (20)	30 (17)	28 (18)
Litter Incidence (%)	11 (65)	16 (80)	11 (61)	12 (75)	16 (80)	13 (65)
Combined						
Fetal Incidence(%)	18 (13)	41 (22)	28 (20)	27 (20)	31 (18)	32 (19)
Litter Incidence (%)	11 (65)	16 (80)	11 (61)	12 (75)	16 (80)	13 (65)

Summary of skeletal malformations and variations

Group	1	2	3	4	5	6
Fetuses Examined	136	185	141	132	175	152
Malformations						
Fetal Incidence(%)	1 (0.7)	1 (0.5)	3 (2.1)	0 (0)	1 (0.6)	1 (0.7)
Litter Incidence (%)	1 (5.9)	1 (5.0)	3 (17)	0 (0)	1 (5.0)	1 (5.0)
Variations						
Fetal Incidence(%)	91 (67)	135 (73)	81 (57)	99 (75)	118 (67)	105 (69)
Litter Incidence (%)	17 (100)	20 (100)	17 (94)	16(100)	19 (95)	20 (100)
Combined						
Fetal Incidence(%)	91 (67)	135 (73)	82 (58)	99 (75)	119 (68)	105 (69)
Litter Incidence (%)	17 (100)	20 (100)	17 (94)	16 (100)	17 (94)	20 (100)

Summary and Evaluation:

1. No significant adverse effects were noted in this study. This is probably due to the relatively low dose of levodopa/carbidopa used in this study. Higher doses should have been used to more fully assess the potential interactions between levodopa and entacapone in this test system.
2. No adverse effects on fetal parameters were observed.

Combination of Entacapone, Levodopa, and Carbidopa: Rat Developmental Toxicity Study

Volume #, and page #: Volume 29, page 1
Study No: and number: [redacted] Study Number 6831-101
Site and testing facility: [redacted]
GLP compliance: Yes
QA- Reports Yes (X) No ( ):
Lot and batch numbers: 032210
Protocol reviewed by Division Yes ( ) No (X):

Methods:

- Species/strain: Rat, Sprague-Dawley Crl:CD BR
- Doses employed:

Group	Dosage Level mg/kg/day	Dosing Schedule Gestation Day	Number Mated Female
1 (Control)	0	6-17	25
2 (Low combination)	40+40+10	6-17	25
3 (Mid combination)	80+40+10	6-17	25
4 (High combination)	600+40+10	6-17	25
5 (Entacapone)	600	6-17	25
6 (Levodopa/Carbidopa)	40+10	6-17	25

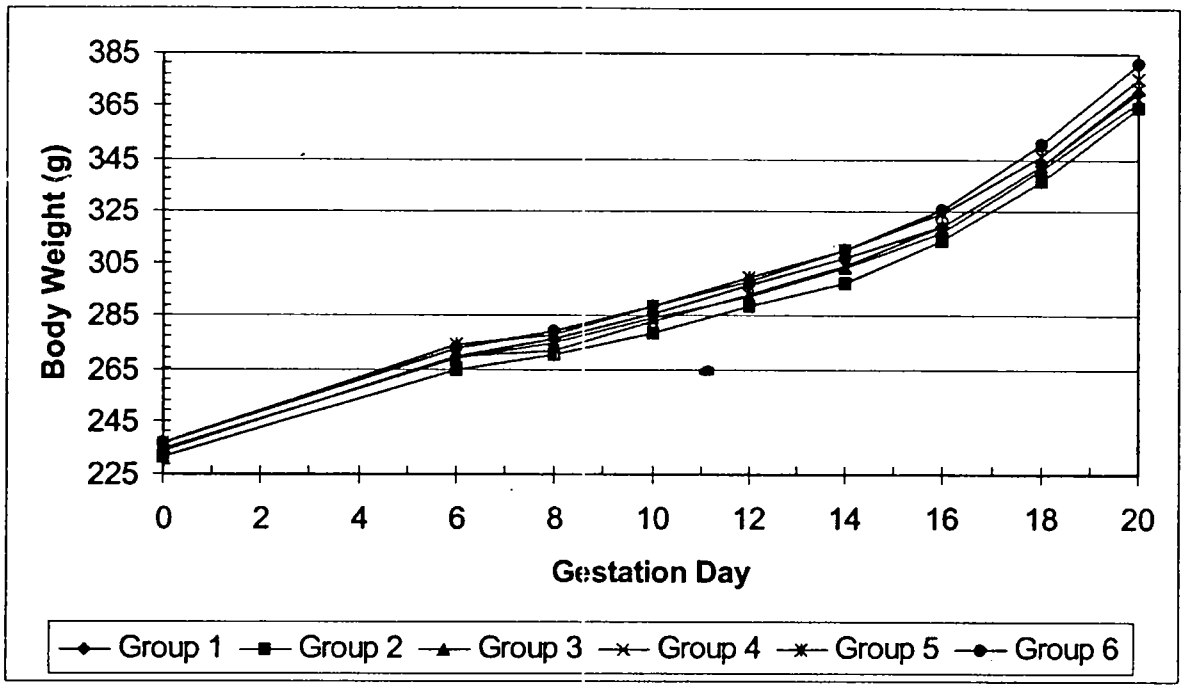
Note: The combinations are entacapone + levodopa + carbidopa

Figure 9, from page 14 of Report 6831-101

- Route of Administration: Oral
- Study Design: Rats dosed GD 6-17
- Number of animals/sex/dosing group: 25 females/group
- Parameters and endpoints evaluated: Fetal toxicity and abnormalities in fetal development
- Statistical evaluations:

Results:

- Clinical signs: None
- Mortality: one group 6 dam on GD 6
- Body weight: Slight decrease in body weight gain from GD 6 to 8 in groups IV and V versus control (2.32, 3.95 versus 6.65 g, respectively); no other effects



- Food consumption: Slight decrease in food consumption from GD 6 to 8 in groups 4 and 5 versus control (18.2, 19.9 versus 22.2 in controls, respectively); no other effects
- Toxicokinetics: Not done
- Embryo-fetal Development
  - In-life observations:
- Terminal and Necroscopic evaluations:
  - Dams: 1 dam in groups 3, 4, and 5 had no viable fetuses

Group	1	2	3	4	5	6
Pregnant Dams	20	18	19	19	19	21
Total Litter Loss	0	0	1	1	1	0
Dams with viable fetuses	20	18	18	18	18	21
Corpora Lutea/dam	14.8	16.4	15.3	16.2	15.1	15.2
Preimplantation loss(%)	3.4 (30)	3.6 (24)	3.4 (28)	3.2 (21)	3.4 (25)	2.7 (20)
Implantation Sites/dam	11.4	12.8	11.9	13.0	11.7	12.5
Early resorptions	0.6	0.7	0.7	0.4	0.6	0.1
Late resorptions	0.1	0.0	0.1	0.0	0.0	0.0
Dead fetuses	0.0	0.0	0.0	0.0	0.0	0.0
Postimplantation loss (%)	0.7 (5.2)	0.7 (4.7)	0.8 (12)	0.4 (9.1)	0.6 (7.4)	0.1 (1.5)
Live Fetuses/dam	10.8	12.2	11.2	12.6	11.2	12.4
Mean Fetal Weight (g)	3.69	3.40	3.59	3.47	3.60	3.54
Adjusted Fetal Weight	3.65	3.40	3.59	3.51	3.59	3.55

- Offspring: No effects

Summary of external malformations and variations

Group	1	2	3	4	5	6
Fetuses Examined	215	219	212	239	212	248
Malformations						
Fetal Incidence(%)	2 (0.9)	1 (0.5)	0 (0)	0 (0)	0 (0)	0 (0)
Litter Incidence (%)	2 (10)	2 (5.6)	0 (0)	0 (0)	0 (0)	0 (0)
Variations						
Fetal Incidence(%)	1 (0.5)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
Litter Incidence (%)	1 (5)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
Combined						
Fetal Incidence(%)	2 (0.9)	1 (0.5)	0 (0)	0 (0)	0 (0)	0 (0)
Litter Incidence (%)	2 (10)	2 (5.6)	0 (0)	0 (0)	0 (0)	0 (0)

Summary of visceral malformations and variations

Group	1	2	3	4	5	6
Fetuses Examined	108	106	105	121	104	123
Malformations						
Fetal Incidence(%)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
Litter Incidence (%)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
Variations						
Fetal Incidence(%)	15 (14)	7 (6.6)	1 (1.0)	8 (6.6)	2 (1.9)	10 (8.1)
Litter Incidence (%)	7 (41)	6 (33)	1 (5.6)	5 (28)	2 (11)	6 (30)
Combined						
Fetal Incidence(%)	15 (14)	7 (6.6)	1 (1.0)	8 (6.6)	2 (1.9)	10 (8.1)
Litter Incidence (%)	7 (41)	6 (33)	1 (5.6)	5 (28)	2 (11)	6 (30)

Summary of skeletal malformations and variations

Group	1	2	3	4	5	6
Fetuses Examined	107	113	107	118	108	125
Malformations						
Fetal Incidence(%)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
Litter Incidence (%)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
Variations						
Fetal Incidence(%)	92 (86)	88 (78)	93 (87)	96 (81)	95 (88)	113 (90)
Litter Incidence (%)	19 (100)	18 (100)	16 (94)	18 (100)	17 (94)	10 (100)
Combined						
Fetal Incidence(%)	92 (86)	88 (78)	93 (87)	96 (81)	95 (88)	113 (90)
Litter Incidence (%)	19 (100)	18 (100)	16 (94)	18 (100)	17 (94)	10 (100)

Key Study findings:

1. No adverse effects were observed on maternal or fetal parameters. Higher doses should have been used.

GENETIC TOXICOLOGY

The following section is excerpted from Dr. T.D. Steele's NDA Review of entacapone (NDA 20-796).

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

Volume #, and page #: Volume 31, page 1

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.

Figure 10, from page 56 of Dr. T.D. Steele Review of Entacapone (NDA 20-796)

Entacapone: Induction of Micronuclei in the Bone Marrow of Treated Mice in the Presence of Carbidopa and L-Dopa

Volume #, and page #: Volume 31, page 65

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

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.

Figure 11, from page 57 of Dr. T.D. Steele Review of Entacapone (NDA 20-796)

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.

Figure 12, from page 58 of Dr. T.D. Steele Review of Entacapone (NDA 20-796)

APPEARS THIS WAY
ON ORIGINAL

Entacapone: Induction of Micronuclei in the Bone Marrow of Treated Mice in the Presence of Carbidopa and L-Dopa

Study no: 544/60-D6172
Study type (if not reflected in title):
Volume #, and page #: Volume 31 / Page 120
Conducting laboratory and location: [redacted]

Date of study initiation: September 26, 2000
GLP compliance: Yes
QA reports: yes (X) no ( )
Drug, lot #, radiolabel, and % purity: Entacapone Batch 134, 99.5% pure
Carbidopa, Batch 010, 99.3% pure
Levodopa, Batch 002, 99.2% pure

Formulation/vehicle: 0.5% methyl cellulose aqueous suspension (all drug products)

Methods:

Strains/species/cell line: Mouse, CD-1
Dose selection criteria:
Basis of dose selection: Maximum tolerated dose in range finding study
Range finding studies: significant mortality was observed in mice dosed at 2000 mg/kg entacapone in combination with levodopa/carbidopa (5/17 males and 3/18 female mice died within 24 hours). No deaths occurred at 1400 mg/kg entacapone in combination with levodopa/carbidopa.
Test agent stability: Stable
Metabolic activation system: Not applicable
Controls:
Vehicle:
Negative controls:
Positive controls: Cyclophosphamide
Comments: Reference control of levodopa/carbidopa alone included
Exposure conditions:
Incubation and sampling times: 24 and 48 hours post dosing
Doses used in definitive study:

Negative control
240 mg/kg levodopa + 60 mg/kg carbidopa
371 mg/kg entacapone + 240 mg/kg levodopa + 60 mg/kg carbidopa
700 mg/kg entacapone + 240 mg/kg levodopa + 60 mg/kg carbidopa
1400 mg/kg entacapone + 240 mg/kg levodopa + 60 mg/kg carbidopa
40 mg/kg cyclophosphamide

Study design:
Analysis:
No. of replicates: 2
Counting method: Blinded
Criteria for positive results:

Acceptance criteria

The assay is considered valid if the following criteria are met

1. the incidence of micronucleated PCE in the vehicle control group falls within or close to the historical vehicle control range as given in Appendix 6, and

2. at least five animals/sex out of each group at each kill time are available for analysis, and

3. the positive control chemical (CPA) induced a statistically significant increase in the frequency of micronucleated PCE

Evaluation criteria

A test article is considered as positive in this assay if

1. a statistically significant increase in the frequency of micronucleated PCE occurs at least at one dose, and

2. the frequency of micronucleated PCE at such a point exceeds the historical vehicle control range.

Figure 13, from page 21 of Report 544/60-D6172

Summary of individual study findings:

Study validity: The negative control values were within the historical control range. The positive control induced a significant increase in micronucleated PCE cells.

Study outcome:

The Levodopa/carbidopa combination was negative in this study as was the combination of entacapone with levodopa/carbidopa.

Table 1 - Male animals, 24 hour time point

Treatment group (mg/kg)	Carbidopa/L-dopa (60 and 240 mg/kg respectively)	Mean ratio PCE/NCE	Group mean frequency of micronucleated PCE ± SD (per 1000 cells)
Negative control (Vehicle)	-	1.45	0.58 ± 0.79
Reference article control (Vehicle)	+	0.92	0.17 ± 0.26
Entacapone (371 mg/kg)	+	1.07	0.25 ± 0.41
Entacapone (700 mg/kg)	+	1.13	0.50 ± 0.32
Entacapone (1400 mg/kg)	+	0.78	0.20 ± 0.27
CPA (40 mg/kg)	-	1.01	9.59 ± 3.84

SD standard deviation
- Animals not dosed with reference articles (Carbidopa/L-dopa)
+ Animals also received reference articles (Carbidopa/L-dopa)

Table 2 - Female animals, 24 hour time point

Treatment group (mg/kg)	Carbidopa/L-dopa (60 and 240 mg/kg respectively)	Mean ratio PCE/NCE	Group mean frequency of micronucleated PCE ± SD (per 1000 cells)
Negative control (Vehicle)	-	0.94	0.41 ± 0.38
Reference article control (Vehicle)	+	1.00	0.25 ± 0.27
Entacapone (371 mg/kg)	+	1.23	0.17 ± 0.26
Entacapone (700 mg/kg)	+	1.08	0.58 ± 0.37
Entacapone (1400 mg/kg)	+	1.03	0.41 ± 0.58
CPA (40 mg/kg)	-	0.92	2.89 ± 5.75

SD standard deviation
- Animals not dosed with reference articles (Carbidopa/L-dopa)
+ Animals also received reference articles (Carbidopa/L-dopa)

Figure 14, from page 28 of Report 544/60-D6172

Table 3 - Male animals, 48 hour time point

Treatment group (mg/kg)	Carbidopa/L-dopa (60 and 240 mg/kg respectively)	Mean ratio PCE/NCE	Group mean frequency of micronucleated PCE ± SD (per 1000 cells)
Negative control (Vehicle)	-	0.9	0.25 ± 0.42
Reference article control (Vehicle)	+	1.0	0.10 ± 0.22
Entacapone (371 mg/kg)	+	0.8	0.17 ± 0.41
Entacapone (700 mg/kg)	+	0.9	0.33 ± 0.60
Entacapone (1400 mg/kg)	+	0.8	0.33 ± 0.60
CPA (40 mg/kg)*	-	0.9	14.26 ± 6.69

SD *standard deviation*
- Animals not dosed with reference articles (Carbidopa/L-dopa)
+ Animals also received reference articles (Carbidopa/L-dopa)
* Animals dosed 24 hours before sampling

Table 4 - Female animals, 48 hour time point

Treatment group (mg/kg)	Carbidopa/L-dopa (60 and 240 mg/kg respectively)	Mean ratio PCE/NCE	Group mean frequency of micronucleated PCE ± SD (per 1000 cells)
Negative control (Vehicle)	-	1.03	0.33 ± 0.26
Reference article control (Vehicle)	+	1.01	0.33 ± 0.41
Entacapone (371 mg/kg)	+	0.78	0.33 ± 0.41
Entacapone (700 mg/kg)	+	0.97	0.33 ± 0.26
Entacapone (1400 mg/kg)	+	0.94	0.43 ± 0.49

SD standard deviation
- Animals not dosed with reference articles (Carbidopa/L-dopa)
+ Animals also received reference articles (Carbidopa/L-dopa)

Figure 15, from page 29 of Report 544/60-D6172

Carbidopa Related Impurities: Reverse Mutation in Five Histidine-Requiring Strains of Salmonella typhimurium

Study no: **544/71-D6171**
Study type (if not reflected in title):
Volume #, and page #: **Volume 1.33 / Page 1**
Conducting laboratory and location: [redacted]

Date of study initiation: **November 13, 2001**
GLP compliance: **Yes**
QA reports: yes (X) no ( )
Drug, lot #, radiolabel, and % purity: pure carbidopa Q339/P21 / carbidopa spiked with impurities (see below) Q339/P30

Impurity	Pure carbidopa	Carbidopa with impurities
[redacted]		

[redacted] and [redacted] are [redacted] impurities of carbidopa
[redacted] is limit of quantification

Figure 16, from page 13 of Report 544/71-D6171

Formulation/vehicle: **0.1 M hydrochloric acid**

Methods:

Strains/species/cell line: Salmonella typhimurium TA98, TA100, TA102, TA1535, TA1537
Dose selection criteria:
Basis of dose selection: cytotoxicity at 5000 ug/ml
Range finding studies:
Test agent stability:
Metabolic activation system: Aroclor induced male Sprague-Dawley rat hepatic S9
Controls:
Vehicle: Yes
Negative controls: Yes
Positive controls:

Chemical	Source	Stock * concentration (µg/mL)	Final concentration (µg/plate)	Use Strain(s)	S-9
2-nitrofluorene (2NF)	[redacted]	50	5.0	TA98	-
Sodium azide (NaN_3)	[redacted]	20	2.0	TA100, TA1535	-
9-aminoacridine (AAC)	[redacted]	500	50.0	TA1537	-
Glutaraldehyde (GLU)	[redacted]	250	25.0	TA102	-
Benzo[a]pyrene (B[a]P)	[redacted]	100	10.0	TA98	+
2-aminoanthracene (AAN)	[redacted]	50	5.0	TA100, TA1535, *TA1537*	+
		200	20.0	TA102	+

* With the exception of NaN_3 and GLU, which were prepared in water, all stock solutions were prepared in sterile anhydrous analytical grade dimethyl sulphoxide (DMSO). All stock solutions were stored in aliquots at 1-10°C in the dark, with the exception of B[a]P which was stored in aliquots at -80°C in the dark.

Figure 17, from page 15 of Report 544/71-D6171

Comments:
Exposure conditions:
Incubation and sampling times: 72 hours
Doses used in definitive study:

Experiment	S-9	Volume additions (mL)	Concentration of treatment solution (mg/mL)	Final concentration (µg/plate)
Range-finder Experiment	- and +	0.5	0.0032	1.6
			0.016	8
			0.08	40
			0.40	200
			2.00	1000
			10.00	5000
Mutation Experiment 1	- and +	0.2	0.15625	3.125
			0.0625	12.5
			0.25	50
			1.00	200
			4.00	800
			16.00	3200
Mutation Experiment 1	- and +	0.31	4.00	1240
			16.00	4960
Mutation Experiment 2	- and +	0.31	0.2268	70.31
			0.4729	146.6
			0.9074	281.3
			1.815	562.5
			3.629	1125
			7.258	2250
			14.52	4500

Figure 18, from page 14 of Report 544/71-D6171

Study design:
Analysis:
No. of replicates: 3 replicates/dose
Counting method: [redacted]
Criteria for positive results:

Acceptance criteria
The assay was considered valid if the following criteria were met:

1. the mean negative control counts fell within the normal ranges as defined in Appendix 4

2. the positive control chemicals induced clear increases in revertant numbers confirming discrimination between different strains, and an active S-9 preparation

3. no more than 5% of the plates were lost through contamination or some other unforeseen event.

In addition, as untreated control treatments were employed, solvent and untreated control data were compared in order to confirm the acceptability/compatibility of the solvent and dose volumes employed with this assay system.

Evaluation criteria
The test articles were considered to be mutagenic if:

1. the assay was valid (see above)

2. Dunnett's test gave a significant response ($p \leq 0.01$) and the data set(s) showed a significant dose correlation

3. the positive responses described above were reproducible.

Figure 19, from page 19 of Report 544/71-D6171

Summary of individual study findings:

Study validity: The positive and negative controls gave appropriate results.

Study outcome:

Carbidopa increased the incidence of mutations in TA1535 and TA1537. The presence of the impurity did not appear to affect the mutagenicity of carbidopa. Sporadic increases in revertants were observed TA98 and TA100 strains.

Pure carbidopa: summary of mean revertant colonies (-S-9) - Experiment 1

Substance	Dose Level µg/plate	TA98	TA100	TA1535	TA1537	TA102
		Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD
0.1 M HCl	100 µl	28 ± 6	81 ± 21	7 ± 3	10 ± 4	327 ± 51
Untreated	-	23 ± 8	129 ± 20	16 ± 3	9 ± 2	365 ± 6
Pure carbidopa	3.125	23 ± 11	117 ± 14	14 ± 7	16 ± 11	298 ± 7
	12.5	27 ± 10	101 ± 27	13 ± 2	8 ± 1	340 ± 33
	50	13 ± 1	101 ± 20	12 ± 4	9 ± 6	376 ± 17
	200	22 ± 5 (S)	123 ± 6 (S)	19 ± 5	23 ± 2	382 ± 17 (S)
	800	12 ± 7 (V)	118 ± 30 (V+M)	19 ± 9 (S)	49 ± 6 (S)	185 ± 68 (S)
	3200	- (T)	- (T)	- (T)	- (T)	237 ± 79 (S)
Positive Controls	Compound	2NF	NaN3	NaN3	AAC	GLU
	Dose Level	5 µg	2 µg	2 µg	50 µg	25 µg
	Mean ± SD	1185 ± 34	490 ± 29	551 ± 43	176 ± 85	848 ± 23

SD Standard deviation

2NF 2-Nitrofluorene
NaN3 Sodium azide
AAC 9-Aminoacridine
GLU Glutaraldehyde

S : Slight thinning of background lawn
T : Toxic, no revertant colonies
V : Very thin background lawn
M : Plate counted manually

Figure 20, from page 33 of Report 544/71-D6171

Carbidopa with impurities: summary of mean revertant colonies (-S-9) - Experiment 1

Substance	Dose Level µg/plate	TA98	TA100	TA1535	TA1537	TA102
		Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD
0.1 M HCl	100 µl	28 ± 6	81 ± 21	7 ± 3	10 ± 4	327 ± 51
Untreated	-	23 ± 8	129 ± 20	16 ± 3	9 ± 2	365 ± 6
Carbidopa with impurities	3.125	34 ± 5	80 ± 8	15 ± 5	9 ± 2	335 ± 33
	12.5	34 ± 3	77 ± 11	19 ± 3	10 ± 1	325 ± 8
	50	37 ± 13	76 ± 8	18 ± 5	13 ± 3	335 ± 18
	200	40 ± 5	135 ± 12	20 ± 4	19 ± 4	364 ± 29
	800	32 ± 3 (S)	146 ± 14	18 ± 9	33 ± 9 (S)	370 ± 24
	3200	18 ± 8 (V)	121 ± 20 (S)	34 ± 2	21 ± 4 (S)	356 ± 33
Positive controls	Compound	2NF	NaN3	NaN3	AAC	GLU
	Dose Level	5 µg	2 µg	2 µg	50 µg	25 µg
	Mean ± SD	1185 ± 34	490 ± 29	551 ± 43	176 ± 85	848 ± 23

SD Standard deviation

2NF 2-Nitrofluorene
NaN3 Sodium azide
AAC 9-Aminoacridine
GLU Glutaraldehyde

S : Slight thinning of background lawn
V : Very thin background lawn

Figure 21, from page 57 of Report 544/71-D6171

Pure carbidopa: summary of mean revertant colonies (+S-9) - Experiment 1

Substance	Dose Level µg/plate	TA98	TA100	TA1535	TA1537	TA102
		Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD
0.1 M HCl	200 µl	31 ± 9	100 ± 8	23 ± 4	16 ± 4	370 ± 32
Untreated	-	37 ± 10	123 ± 12	17 ± 1	22 ± 5	423 ± 31
Pure carbidopa	3.125	31 ± 9	98 ± 8	27 ± 5	15 ± 2	341 ± 42
	12.5	38 ± 6	109 ± 8	21 ± 4	12 ± 4	355 ± 33
	50	28 ± 5	103 ± 1	27 ± 2	16 ± 9	302 ± 46
	200	24 ± 5	112 ± 10	25 ± 7	16 ± 6	324 ± 27 (S)
	800	43 ± 11 (S)	130 ± 10 (S)	30 ± 3	18 ± 2	344 ± 33 (S)
	3200	25 ± 7 (S)	123 ± 14 (S)	31 ± 6	12 ± 1	302 ± 37 (S)
Positive Controls	Compound	BaP	AAN	AAN	AAN	AAN
	Dose Level	10 µg	5 µg	5 µg	5 µg	20 µg
	Mean ± SD	393 ± 42	1659 ± 385	258 ± 49	268 ± 37	2594 ± 97

SD Standard deviation S : Slight thinning of background lawn

BaP Benzo(a)pyrene
AAN 2-Aminoanthracene

Figure 22, from page 34 of Report 544/71-D6171

Carbidopa with impurities: summary of mean revertant colonies (+S-9) - Experiment 1

Substance	Dose Level µg/plate	TA98	TA100	TA1535	TA1537	TA102
		Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD
0.1 M HCl	200 µl	31 ± 9	100 ± 8	23 ± 4	16 ± 4	370 ± 32
Untreated	-	37 ± 10	123 ± 12	17 ± 1	22 ± 5	423 ± 31
Carbidopa with impurities	3.125	27 ± 7	101 ± 10	26 ± 1	12 ± 2	357 ± 17
	12.5	28 ± 4	102 ± 6	25 ± 2	20 ± 2	393 ± 18
	50	36 ± 2	100 ± 8	15 ± 4	12 ± 5	340 ± 14
	200	39 ± 6	98 ± 3	16 ± 0	12 ± 3	335 ± 23 (S)
	800	26 ± 11	112 ± 6	24 ± 6	15 ± 5	380 ± 31 (S)
	3200	20 ± 7 (S)	116 ± 18	31 ± 8	15 ± 5	285 ± 27 (S)
Positive controls	Compound	BaP	AAN	AAN	AAN	AAN
	Dose Level	10 µg	5 µg	5 µg	5 µg	20 µg
	Mean ± SD	393 ± 42	1659 ± 385	258 ± 49	268 ± 37	2594 ± 97

SD Standard deviation

BaP Benzo(a)pyrene
AAN 2-Aminoanthracene

S : Slight thinning of background lawn

Figure 23, from page 58 of Report 544/71-D6171

Pure carbidopa: summary of mean revertant colonies (-S-9) - Experiment 1

Substance	Dose Level µg/plate	TA98	TA100	TA1535	TA1537	TA102
		Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD
0.1 M HCl	310 µl	30 ± 6	87 ± 4	24 ± 5	12 ± 7	280 ± 22
Untreated	-	23 ± 8	129 ± 20	16 ± 3	9 ± 2	365 ± 6
Pure carbidopa	1240	64 ± 8	192	36 ± 9	21 ± 4	347 ± 10 (S)
	4960	66 ± 28 (S)	- (T)	64 ± 14	16 ± 3 (S)	260 ± 30 (S)
Positive controls	Compound	2NF	NaN_3	NaN_3	AAC	GLU
	Dose Level	5 µg	2 µg	2 µg	50 µg	25 µg
	Mean ± SD	1185 ± 34	690 ± 29	551 ± 45	176 ± 85	848 ± 23

SD Standard deviation

2NF 2-Nitrofluorene
NaN_3 Sodium azide
AAC 9-Aminoacridine
GLU Glutaraldehyde

S : Slight thinning of background lawn
T : Toxic, no revertant colonies

Figure 24, from page 45 of Report 544/71-D6171

Carbidopa with impurities: summary of mean revertant colonies (-S-9) - Experiment 1

Substance	Dose Level µg/plate	TA98	TA100	TA1535	TA1537	TA102
		Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD
0.1 M HCl	310 µl	30 ± 6	87 ± 4	24 ± 5	12 ± 7	280 ± 22
Untreated	-	23 ± 8	129 ± 20	16 ± 3	9 ± 2	365 ± 6
Carbidopa with impurities	1240	30 ± 12 (S)	144 ± 10 (S)	34 ± 10	26 ± 11 (S)	292 ± 16 (S)
	4960	27 ± 24 (S)	105 ± 15 (S)	74 ± 12	15 ± 13 (S)	197 ± 8 (S)
Positive controls	Compound	2NF	NaN_3	NaN_3	AAC	GLU
	Dose Level	5 µg	2 µg	2 µg	50 µl	25 µg
	Mean ± SD	1185 ± 34	690 ± 29	551 ± 45	176 ± 85	848 ± 23

SD Standard deviation

2NF 2-Nitrofluorene
NaN_3 Sodium azide
AAC 9-Aminoacridine
GLU Glutaraldehyde

S : Slight thinning of background lawn

Figure 25, from page 69 of Report 544/71-D6171

Pure carbidopa: summary of mean revertant colonies (+S-9) - Experiment 1

Substance	Dose Level µg/plate	TA98	TA100	TA1535	TA1537	TA102
		Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD
0.1 M HCl	310 µl	31 ± 5	99 ± 13	20 ± 2	12 ± 3	355 ± 24
Untreated	-	37 ± 10	123 ± 12	17 ± 1	22 ± 5	423 ± 31
Pure carbidopa	1240	25 ± 2	103 ± 13	30 ± 9	11 ± 2	312 ± 12
	4960	35 ± 4	99 ± 6	39 ± 24	6 ± 2	165 ± 24 (S)
Positive controls	Compound	B[a]P	AAN	AAN	AAN	AAN
	Dose Level	10 µg	5 µg	5 µg	5 µg	20 µg
	Mean ± SD	393 ± 42	1659 ± 360	258 ± 49	268 ± 37	2594 ± 97

SD Standard deviation

B[a]P Benzo[a]pyrene
AAN 2-Aminoanthracene

S : Slight thinning of background lawn

Figure 26, from page 46 of Report 544/71-D6171

Carbidopa with impurities: summary of mean revertant colonies (+S-9) - Experiment 1

Substance	Dose Level µg/plate	TA98	TA100	TA1535	TA1537	TA102
		Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD
0.1 M HCl	310 µl	31 ± 5	99 ± 13	20 ± 2	12 ± 3	355 ± 24
Untreated	-	37 ± 10	123 ± 12	17 ± 1	22 ± 5	423 ± 31
Carbidopa with impurities	1240	33 ± 6	140 ± 13	21 ± 16	9 ± 4	314 ± 34 (S)
	4960	24 ± 16	128 ± 4	60 ± 9	5 ± 1	176 ± 90 (S)
Positive controls	Compound	B[a]P	AAN	AAN	AAN	AAN
	Dose Level	10 µg	5 µg	5 µg	5 µg	20 µg
	Mean ± SD	393 ± 42	1659 ± 360	258 ± 49	268 ± 37	2594 ± 97

SD Standard deviation

B[a]P Benzo[a]pyrene
AAN 2-Aminoanthracene

S : Slight thinning of background lawn

Figure 27, from page 70 of Report 544/71-D6171

Pure carbidopa: summary of mean revertant colonies (-S-9) - Experiment 2

Substance	Dose Level µg/plate	TA98	TA100	TA1535	TA1537	TA102
		Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD
0.1 M HCl	100 µl	27 ± 11	89 ± 26	15 ± 6	6 ± 2	298 ± 17
Untreated	-	31 ± 12	119 ± 16	13 ± 6	13 ± 6	314 ± 33
Pure carbidopa	70.31	32 ± 3	93 ± 7	17 ± 4	5 ± 1	388 ± 41
	140.6	34 ± 12	111 ± 12	16 ± 6	12 ± 5	388 ± 35
	281.3	31 ± 10	105 ± 9	14 ± 3	10 ± 6	382 ± 27
	562.5	33 ± 19	125 ± 25	19 ± 8	22 ± 3	386 ± 33
	1125	38 ± 7 (d)	129 ± 18	26 ± 11	21 ± 7 (d)	342 ± 30 (d)
	2250	30 ± 3 (d)	122 ± 13 (d)	29 ± 1	14 ± 3 (d)	450 ± 97 (d)
	4500	- (T)	46 ± 35 (T)	17 ± 12 (d)	- (T)	- (T)
Positive controls	Compound	2NF	NaN3	NaN3	AAC	GLU
	Dose Level	5 µg	2 µg	2 µg	50 µg	25 µg
	Mean ± SD	1168 ± 31	696 ± 15	536 ± 90	153 ± 54	884 ± 30

SD Standard deviation

2NF 2-Nitrofluorene
NaN3 Sodium azide
AAC 9-Aminoacridine
GLU Glutaraldehyde

s : Slight thinning of background lawn
T : Toxic, no revertant colonies
V : Very thin background lawn

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Figure 28, from page 81 of Report 544/71-D6171

Carbidopa with impurities: summary of mean revertant colonies (-S-9) - Experiment 2

Substance	Dose Level µg/plate	TA98	TA100	TA1535	TA1537	TA102
		Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD
0.1 M HCl	100 µl	27 ± 11	89 ± 26	15 ± 6	6 ± 2	298 ± 17
Untreated	-	31 ± 12	119 ± 16	13 ± 6	13 ± 6	314 ± 33
Carbidopa with impurities	70.31	42 ± 10	132 ± 18	25 ± 2	13 ± 1	411 ± 15
	140.6	32 ± 4	145 ± 22	19 ± 2	17 ± 3	396 ± 34
	281.3	38 ± 11	141 ± 9	27 ± 10	23 ± 10	417 ± 13
	562.5	53 ± 6	172 ± 25	32 ± 2	24 ± 4	430 ± 13
	1125	52 ± 10	168 ± 12	30 ± 3	30 ± 12	458 ± 45
	2250	69 ± 6	163 ± 26	50 ± 12	16 ± 12	486 ± 12
	4500	73 ± 17 (d)	155 ± 24 (d)	36 ± 27	38 ± 7 (d)	380 ± 62 (d)
Positive controls	Compound	2NF	NaN3	NaN3	AAC	GLU
	Dose Level	5 µg	2 µg	2 µg	50 µg	25 µg
	Mean ± SD	1168 ± 31	696 ± 15	536 ± 90	153 ± 54	884 ± 30

SD Standard deviation

2NF 2-Nitrofluorene
NaN3 Sodium azide
AAC 9-Aminoacridine
GLU Glutaraldehyde

s : Slight thinning of background lawn

Figure 29, from page 93 of Report 544/71-D6171

Pure carbidopa: summary of mean revertant colonies (+S-9) - Experiment 2

Substance	Dose Level mg/plate	TA98	TA100	TA1535	TA1537	TA102
		Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD
0.1 M HCl	100 µl	22 ± 9	93 ± 6	14 ± 6	13 ± 12	306 ± 35
Untreated	-	25 ± 9	131 ± 9	20 ± 5	23 ± 10	359 ± 19
Pure carbidopa	70.31	23 ± 7	78 ± 18	12 ± 6	15 ± 10	340 ± 14
	140.6	31 ± 7	111 ± 24	18 ± 3	12 ± 2	303 ± 17
	281.3	22 ± 6	131 ± 5	13 ± 3	10 ± 2	279 ± 37
	562.5	31 ± 7	93 ± 13	22 ± 6	24 ± 4	405 ± 128
	1125	31 ± 3	127 ± 16	33 ± 2	13 ± 1	425 ± 38
	2250	29 ± 13 (S)	94 ± 33 (S)	31 ± 6 (S)	7 ± 3 (S)	434 ± 10 (S)
	4500	32 ± 5 (V)	125 ± 16 (S)	41 ± 2 (S)	5 ± 3 (S)	- (T)
Positive Controls	Compound	BAP	AAN	AAN	AAN	AAN
	Dose Level	10 µg	5 µg	5 µg	5 µg	20 µg
	Mean ± SD	227 ± 39	2254 ± 229	274 ± 76	291 ± 35	2298 ± 273

SD : Standard deviation
BAP : Benzo(a)pyrene
AAN : 2-Aminoanthracene
S : Slight thinning of background lawn
T : Toxic, no revertant colonies
V : Very thin background lawn

Figure 30, from page 82 of Report 544/71-D6171

Carbidopa with impurities: summary of mean revertant colonies (+S-9) - Experiment 2

Substance	Dose Level mg/plate	TA98	TA100	TA1535	TA1537	TA102
		Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD
0.1 M HCl	100 µl	22 ± 9	93 ± 6	14 ± 6	13 ± 12	306 ± 35
Untreated	-	25 ± 9	131 ± 9	20 ± 5	23 ± 10	359 ± 19
Carbidopa with impurities a	70.31	30 ± 4	107 ± 20	23 ± 10	20 ± 2	373 ± 32
	140.6	31 ± 5	115 ± 27	25 ± 7	19 ± 7	443 ± 41
	281.3	35 ± 6	128 ± 17	31 ± 6	24 ± 8	401 ± 25
	562.5	36 ± 10	128 ± 15	27 ± 2	20 ± 6	377 ± 16
	1125	38 ± 7	142 ± 10	37 ± 8	17 ± 3	423 ± 48
	2250	42 ± 4	137 ± 3	44 ± 11	14 ± 8	446 ± 49
	4500	32 ± 12 (S)	137 ± 14 (S)	34 ± 16	13 ± 6 (S)	288 ± 75 (S)
Positive controls	Compound	BAP	AAN	AAN	AAN	AAN
	Dose Level	10 µg	5 µg	5 µg	5 µg	20 µg
	Mean ± SD	227 ± 39	2254 ± 229	274 ± 76	291 ± 35	2298 ± 273

SD : Standard deviation
BAP : Benzo(a)pyrene
AAN : 2-Aminoanthracene
S : Slight thinning of background lawn

Figure 31, from page 94 of Report 544/71-D6171

Carbidopa Related Impurities: Mutation at the Thymidine Kinase (tk) Locus of mouse Lymphoma L5178Y Cells (MLA) Using the Microtitre Fluctuation Technique

Study no: **544/72-D6173**
Study type (if not reflected in title):
Volume #, and page #: **Volume 33 / Page 112**
Conducting laboratory and location: [redacted]

Date of study initiation: **November 21, 2002**
GLP compliance: **Yes**
QA reports: yes (X) no ( )
Drug, lot #, radiolabel, and % purity: **Extra pure carbidopa Batch Q339/P21; carbidopa spiked with impurities listed below Q339/P30**

Impurity	Pure carbidopa	Carbidopa with impurities
[redacted]		
[redacted]		
[redacted]		

* [redacted] and [redacted] are [redacted] impurities of carbidopa
* [redacted] is limit of quantification

Figure 32, from page 14 of Report 544/72-D6173

Formulation/vehicle: **DMSO**

Methods:

Strains/species/cell line: L5178Y TK+/- mouse lymphoma cells
Dose selection criteria:
Basis of dose selection: limit of solubility
Range finding studies:
Test agent stability:
Metabolic activation system: Aroclor 1254 induced male Sprague-Dawley rat hepatic S9
Controls:
Vehicle:
Negative controls: DMSO treated
Positive controls: 4-nitroquinoline-1-oxide (-S9); benzo(a)pyrene (+S9)
Comments:
Exposure conditions:
Incubation and sampling times: 3 and 24 hours incubation; 48 hour expression
Doses used in definitive study:

Cultures selected for mutation assessment for pure carbidopa and carbidopa with impurities

Range-finder		Experiment 1 (µg/mL)		Experiment 2 (µg/mL)	
- S-9	+ S-9	- S-9	+ S-9	- S-9	+ S-9
0	0	0	0	0	0
3.938	3.938	6.25	6.25	2.5	25
7.875	7.875	12.5	12.5	5	50
15.75	15.75	25	25	10	100
31.5	31.5	50	50	20	150
63	63	100	100	30	200
126	126	200	200	40	
				50	
				60	
		NQO 0.05	BP 2	NQO 0.02	BP 2
		NQO 0.1	BP 3	NQO 0.04	BP 3

Figure 33, from page 21 of Report 544/72-D6173

Study design:
Analysis:
No. of replicates: two/dose
Counting method:
Criteria for positive results:

Acceptance criteria

The assay was considered valid if all the following criteria were met:

1. the mutant frequencies in the negative (solvent) control cultures fell within the normal range (above 60 mutants per 10^6 viable cells but not more than three times the historical mean value)
2. at least one concentration of each of the positive control chemicals induced a clear increase in mutant frequency (the difference between the positive and negative control mutant frequencies was greater than half the historical mean value)
3. the plating efficiencies of the negative controls from the mutation experiments were between the range of 60% to 140% on Day 0 and 70% to 130% on Day 2.

Evaluation criteria

The test article was considered to be mutagenic if all the following criteria were met:

1. the assay was valid (see Acceptance criteria)
2. the mutant frequency at one or more doses was significantly greater than that of the negative control ($p<0.05$)
3. there was a significant dose-relationship as indicated by the linear trend analysis ($p<0.05$).

Figure 34, from page 25 of Report 544/72-D6173

Summary of individual study findings:

Study validity: Criteria for valid study are met.

Study outcome:

Increased mutations after 24 hour exposure in the absence of S9. No effects at other exposure periods. Increase appears to be mostly in large colonies (indicative of gene mutation). Carbidopa impurities did not appear to alter the pattern of genotoxicity for carbidopa in this test.

Pure carbidopa: summary of results

Experiment 1 (3 hour treatment +/- S-9)

Treatment (µg/mL)	-S-9 %RS	RTG	MF§		Treatment (µg/mL)	+S-9 %RS	RTG	MF§	
0	100.00	1.00	74.04		0	100.00	1.00	81.85	
6.25	98.38	0.83	75.88	NS	6.25	99.61	0.97	71.74	NS
12.5	104.87	0.93	86.14	NS	12.5	95.72	0.99	79.85	NS
25	128.71	0.93	80.01	NS	25	93.75	0.94	73.26	NS
50	101.66	0.92	66.71	NS	50	95.84	1.08	90.74	NS
100	108.05	0.88	65.43	NS	100	94.01	0.84	82.01	NS
200	101.74	0.84	77.25	NS	200	94.90	0.94	79.72	NS
Linear trend				NS	Linear trend				NS
NQO					BP				
0.05	88.25	0.83	212.55		2	74.05	0.46	761.13	
0.1	92.09	0.68	292.70		3	39.42	0.29	1042.75	

Experiment 2 (24 hour treatment – S-9, 3 hour treatment + S-9)

Treatment (µg/mL)	-S-9 %RS	RTG	MF§		Treatment (µg/mL)	+S-9 %RS	RTG	MF§	
0	100.00	1.00	74.76		0	100.00	1.00	73.09	
2.5	92.15	0.85	64.80	NS	25	113.14	0.98	75.08	NS
5	96.38	0.76	57.32	NS	50	116.71	1.05	63.21	NS
10	109.07	0.83	66.48	NS	100	102.77	0.99	58.03	NS
20	73.92	0.86	68.48	NS	150	82.02	0.94	81.77	NS
30	56.47	0.82	74.13	NS	200	98.99	0.99	66.00	NS
40	24.09	0.43	150.28	*					
50	8.76	0.18	273.48	*					
60 X	3.09	0.08	339.83						
Linear trend				***	Linear trend				NS
NQO					BP				
0.02	88.61	1.00	178.12		2	81.06	0.68	352.12	
0.04	70.62	0.75	356.63		3	86.66	0.59	568.42	

§ 5-TFT resistant mutants/10^6 viable cells 2 days after treatment
%RS Percent relative survival adjusted by post treatment cell counts
NS Not significant
X Treatment excluded from final test statistics due to excessive toxicity
* Comparison of each treatment with control: Dunnett's test (one-sided), significant at 5% level
*, **, *** Test for linear trend: χ^2 (one-sided), significant at 5%, 1% and 0.1% level respectively

Figure 35, from page 30 of Report 544/72-D6173

Carbidopa with impurities: summary of results

Experiment 1 (3 hour treatment +/- S-9)

Treatment (µg/mL)	-S-9 %RS	RTG	MF§		Treatment (µg/mL)	+S-9 %RS	RTG	MF§	
0	100.00	1.00	74.04		0	100.00	1.00	81.85	
6.25	112.16	0.92	84.72	NS	6.25	108.05	0.99	69.90	NS
12.5	101.03	0.82	84.81	NS	12.5	94.26	1.05	96.12	NS
25	94.96	0.81	79.78	NS	25	76.66	1.12	87.76	NS
50	106.02	1.05	78.36	NS	50	112.96	1.66	62.17	NS
100	100.10	0.90	81.66	NS	100	108.58	0.97	97.54	NS
200	109.85	0.86	75.27	NS	200	98.53	1.26	81.20	NS
Linear trend				NS	Linear trend				NS
NQO					BP				
0.05	89.17	0.93	146.72		2	67.93	0.79	443.69	
0.1	84.69	0.80	189.08		3	78.70	0.68	449.46	

Experiment 2 (24 hour treatment – S-9, 3 hour treatment + S-9)

Treatment (µg/mL)	-S-9 %RS	RTG	MF§		Treatment (µg/mL)	+S-9 %RS	RTG	MF§	
0	100.00	1.00	74.76		0	100.00	1.00	73.09	
2.5	102.40	1.11	53.75	NS	25	93.76	1.19	66.06	NS
5	113.73	0.82	66.00	NS	50	98.53	1.13	93.40	NS
10	110.52	0.97	68.00	NS	100	82.44	1.30	67.22	NS
20	100.65	0.95	77.64	NS	150	112.34	1.16	65.10	NS
30	75.56	0.91	103.46	NS	200	106.92	1.05	79.62	NS
40	41.17	0.60	155.50	*					
50	19.88	0.40	165.07	*					
60 X	8.06	0.23	233.41						
Linear trend				***	Linear trend				NS
NQO					BP				
0.02	114.97	0.90	212.79		2	91.08	0.81	376.58	
0.04	87.84	0.92	336.40		3	60.14	0.56	750.34	

§	5-TFT resistant mutants/10^6 viable cells 2 days after treatment
%RS	Percent relative survival adjusted by post treatment cell counts
$	Not plated for viability / 5-TFT resistance
NS	Not significant
X	Treatment excluded from final test statistics due to excessive toxicity
*	Comparison of each treatment with control: Dunnett's test (one-sided), significant at 5% level
*, **, ***	Test for linear trend: χ^2 (one-sided), significant at 5%, 1% and 0.1% level respectively

Figure 36, from page 31 of Report 544/72-D6173

Carbidopa Related Impurities: Induction of Micronuclei in the Bone Marrow of Treated Mice

Study no: **544/73D6172**
Study type (if not reflected in title):
Volume #, and page #: **Volume 33 / Page 214**
Conducting laboratory and location: [redacted]

Date of study initiation: **November 22 2002**
GLP compliance: **Yes**
QA reports: yes (X) no ( )
Drug, lot #, radiolabel, and % purity: **Extra pure carbidopa Batch Q339/P21; carbidopa spiked with impurities listed below Q339/P30**

Impurity	Pure carbidopa	Carbidopa with impurities
[redacted]		
[redacted]		
[redacted]		

[redacted] and [redacted] are [redacted] impurities of carbidopa.
* [redacted] is limit of quantification

Figure 37, from page 13 of Report 544/73-D6172

Formulation/vehicle:

Methods:

Strains/species/cell line: Mice, CD-1
Dose selection criteria: Maximum recommended dose
Basis of dose selection:
Range finding studies: Yes
Test agent stability:
Metabolic activation system: Not applicable
Controls:
Vehicle: 0.5% methyl cellulose aqueous solution
Negative controls:
Positive controls: cyclophosphamide
Comments:
Exposure conditions:
Incubation and sampling times: mice sacrificed at 24 hours after last dose
Doses used in definitive study: 2000 mg/kg for 2 days

Study design:
Analysis:
No. of replicates: 6 mice/sex per dose
Counting method:
Criteria for positive results:

Acceptance criteria
The assay is considered valid if the following criteria are met:

1. the incidence of micronucleated PCE in the vehicle control group falls within or close to the historical vehicle control range as given in Appendix 6, and

2. at least five animals out of each group are available for analysis, and

3. the positive control chemical (CPA) induced a statistically significant increase in the frequency of micronucleated PCE.

Evaluation criteria
A test article is considered as positive in this assay if:

1. a statistically significant increase in the frequency of micronucleated PCE occurs at least at one dose, and

2. the frequency of micronucleated PCE at such a point exceeds the historical vehicle control range.

Figure 38, from page 19 of Report 544/73-D6172

Summary of individual study findings:

Study validity: Criteria for a valid study were met.

Study outcome:

Neither form of carbidopa displayed any activity in this assay.

Treatment group (mg/kg/day)	Kill Time (hours)	Mean Ratio PCE/NCE	Group mean frequency of micronucleated PCE (per 1000 cells) per treatment group (±sd)
Vehicle Control	24	0.95	0.08 ± 0.20
Pure carbidopa (2000)	24	0.95	0.08 ± 0.20
Carbidopa with impurities (2000)	24	0.86	0.08 ± 0.20
CPA, 40+	24	0.85	9.80 ± 4.56

\+ Administered as a single dose
sd Standard deviation.

Figure 39, from page 26 of Report 544/73-D6172

OVERALL SUMMARY AND EVALUATION

Introduction

Parkinson's disease is a neurodegenerative disease characterized by a loss of dopamine fibers. To alleviate the symptoms of decreased dopaminergic tone, levodopa is administered to patients with Parkinson's disease. Levodopa crosses the blood brain barrier where it is converted to dopamine alleviating some of the symptoms. Dopamine is ineffective in Parkinson's disease because it does not cross the blood brain barrier. To increase the efficacy of levodopa, it is given with carbidopa (the combination is called sinemet). Carbidopa inhibits Aromatic-L-Amino Acid Decarboxylase, which breaks down levodopa in the peripheral circulation. This inhibition leads to higher blood levels of levodopa. Another approach to increase the efficacy of levodopa is to prolong the action of dopamine in the brain by inhibiting its breakdown by catechol-O-methyl transferase (COMT). Entacapone is a COMT inhibitor which is approved as adjunctive therapy with sinemet for Parkinson's disease. The drug product in this application combines all three of these approved drugs (entacapone, levodopa, carbidopa) into a single tablet.

Safety Evaluation

Studies originally submitted to NDA 20-796

The preclinical issues of the safety of entacapone in combination with Sinemet was previously examined by Dr. Tom .D. Steele in his review of the original entacapone NDA (NDA 20-796, approved). The following excerpt from his review summarizes his evaluation of the preclinical studies on the interaction of entacapone and Sinemet.

> 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.

Figure 40, from page 97 of Dr. T.D. Steele Review of Entacapone (NDA 20-796)

New Preclinical Studies

Since entacapone is already approved to be administered in conjunction with sinemet, no additional preclinical studies were requested except for an additional in vivo micronucleus test using the combination product. This test was requested because the doses used in the original combination mouse micronucleus test were relatively low. The sinemet dose (40/10 mg/kg levodopa/carbidopa) was lower than the maximum tolerated dose in mice. The combination of entacapone and sinemet was inactive in the in vivo micronucleus test. The doses used (up to 1400 mg/kg entacapone, 240 mg/kg levodopa and 60 mg/kg carbidopa combined) were adequate to adress the potential in vivo genotoxicity of the drug product. Both entacapone and sinemet are genotoxic in the in vitro test systems, so no additional data were required on the combination product.

The primary new preclinical issue was the identification of new impurities [redacted] in addition, a degradation product [redacted] was observed (see Figure 41). Under ICH and FDA guidelines (ICH Q3B Impurities in New Drug Products), it is recommended that a general toxicity study between 2 and 13 weeks in duration is needed to ensure that the new impurity does not add to the toxicity of the drug product. In addition, genotoxicity studies (bacterial reverse mutation assay and in vitro mammalian chromosomal aberration studies) are recommended. The sponsor has conducted a four week toxicity study on carbidopa as well as the genotoxicity battery. The toxicity of the carbidopa spiked with impurities was comparable to the pure carbidopa. In addition, the activity of the spiked carbidopa was similar to that of pure carbidopa in the genotoxicity battery. The degradation product was present in sufficient quantities so that additional studies were not needed. These results suggest that the impurities do not contribute any additional toxicity to the carbidopa drug product.

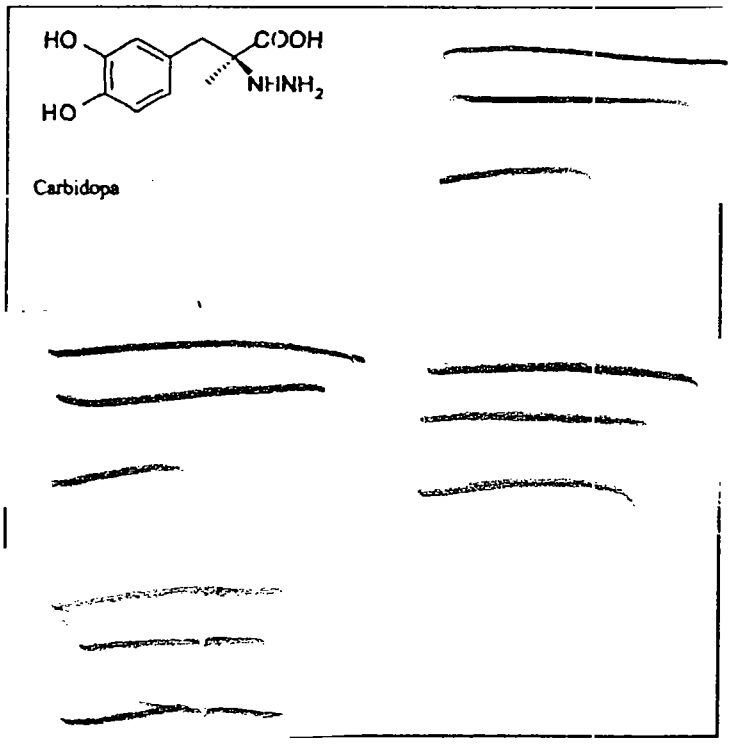

Figure 41, Chemical structures of carbidopa and its impurities and degradation product.

The sponsor submitted two segment two reproductive toxicity studies on the combination of entacapone and sinemet (requested by European authorities). The high doses in the rat study were 600 mg/kg entacapone and 40/10 mg/kg levodopa/carbidopa. The high doses in the rabbit study were 150 mg/kg entacapone and 40/10 mg/kg levodopa/carbidopa. No maternotoxic or fetotoxic effects were observed. The doses used in these studies are below those doses which were tested in previous segment II studies on the individual agents (1000 and 300 mg/kg entacapone in rats and rabbits, respectively; 125/12.5 and 250/25 mg/kg levodopa/carbidopa in rats and rabbits, respectively). Higher doses, especially of sinemet, could have been used. Thus, these studies do not provide much information on how the combination of these drugs may affect embryo-fetal development. Since higher doses of both entacapone and sinemet are known to affect embryo-fetal development in rats and rabbits, it is reasonable to anticipate that higher doses of the combination of these drugs would affect embryo-fetal development.

Labeling Review

The label consists of a combination of the Comtan and Sinemet labels. This positive results for carbidopa in the genotoxicity studies should be added. Since the text has been approved in other drug products, it is acceptable.

RECOMMENDATIONS

Entacapone is an approved drug product which is intended to be used in combination with Sinemet (levodopa/carbidopa) in the treatment of Parkinson's Disease. The present drug product combines entacapone with Sinemet in a single tablet. As such, there are no additional preclinical concerns as to the approval of the product in itself. The sponsor has identified additional impurities in the carbidopa portion of the drug product. The qualification studies were appropriate and did not identify any additional preclinical concerns. **Based on these considerations, this application can be approved from a preclinical perspective.**

Reviewer signature/team leader signature [Concurrence/Non-concurrence]

Paul Roney

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/s/

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