C.3.b. 12-Month Oral Toxicity and Toxicokinetic Study in Dogs

GLP | Research Report: B-161,825 | Sponsor Volumes: 1.27-1.28

Conducted by : F. Hoffmann-LaRoche, Ltd.
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Basel, Switzerland

Summary:

Tolcapone was administered orally in a gelatin capsule at doses of 0, 10, 40 and 150 (2 x 75) mg/kg/day to beagle dogs (4 or 5/sex/dose) for 12 months (the high dose was increased to 180 mg/kg/day for the second half of the study). One dog/sex in the control and HD groups were allowed to recover for 8 weeks.

Vomiting and diarrhea occurred at the mid and high doses. Mean decreases in erythrocyte parameters (RBCs, Hb, PCV, MCV, MCH) were observed in high-dose males at 9 and 12 months. Analysis if the individual data revealed only one animal with moderate decreases, which recovered after eight weeks. Statistically significant increases in bilirubin occurred in MDM and HDM on day 276. The magnitude of the increase was relatively small, and probably not of toxicological significance, although it coincided with a reduction in red blood cells (the sponsor conducted a study which suggested that the bilirubin increase was due to an artifact). Statistically significant increases in relative liver (24%) and kidney weights (22%) in HDM were not accompanied by any histopathological changes.

Increases in plasma exposure to tolcapone were approximately dose proportional, and no gender differences were apparent. Some evidence of drug accumulation was noted. Low levels of the 3-O-methyl metabolite of tolcapone were detected. Relative to plasma exposures in humans receiving the projected maintenance dose of 200 mg, t.i.d. (AUC_{0-24} = 80 µg.hr/ml), tolcapone exposures in dogs were:

LD: below human exposures
MD: 1.0 - 2.0 times the human exposures
HD: 4.5 - 8.2 "

Toxicological findings in 6-month (hyperemia of the stomach mucosa in HD animals) and one month studies (epithelial cells in urine in MD and HD animals) were not evident in this study, despite the use of similar dosage levels.

Methods:

Dosages: 0, 10, 40, 150 (or 180) mg/kg/day (Lot G PUL 606 090)
The high dose (2 x 75 mg/kg administered 5 hrs apart) was selected as the MTD based on a six-month study in which vomiting and diarrhea occurred at this level. It was increased to 180 mg/kg/day for the second half of the study.

Route of Administration: oral in gelatin capsules.

Species/Number: Beagle dogs (males: 8.4 - 11.6 kg; females: 7.2 to 10.0 kg)
4/sex/group except control and HD, which had an additional animal to assess recovery at 8 weeks.

Food Intake: restricted to 5 hrs per day

Results:

Mortality: No clearly drug-related deaths occurred. One HDF died after "misapplication" of the first dose. The replacement for this animal died on day 8 after losing weight. At autopsy, large kidney and bladder stones were evident. Because of the short duration of drug treatment, it is unlikely that the stones were drug-related. A second HDF died due to "misapplication" in month 4.

Clinical Signs:
LD: no significant effects
MD: occasional vomiting and soft feces
HD: more severe and frequent vomiting and diarrhea

Body Weight Gain: A moderate reduction in the second six months was suggested to be due to the increase in dosage (Sponsor Figure 1).

Ophthalmology: (beginning and end of study)

No treatment-related effects.

Hematology: (predose and months 2, 6, 9, and 12)

There were occasional modest, but statistically significant changes in male treatment groups:

decrease RBC	-	HDM (day 276, 367)
decrease Hb	-	HDM (day 276, 367)
decrease PCV	-	HDM (day 276, 367)
decrease MCV	-	HDM (day 367)
decrease MCH	-	HDM (day 367)
increase platelets	-	HDM (day 276, 367)
increase PT	-	HDM (day 367)

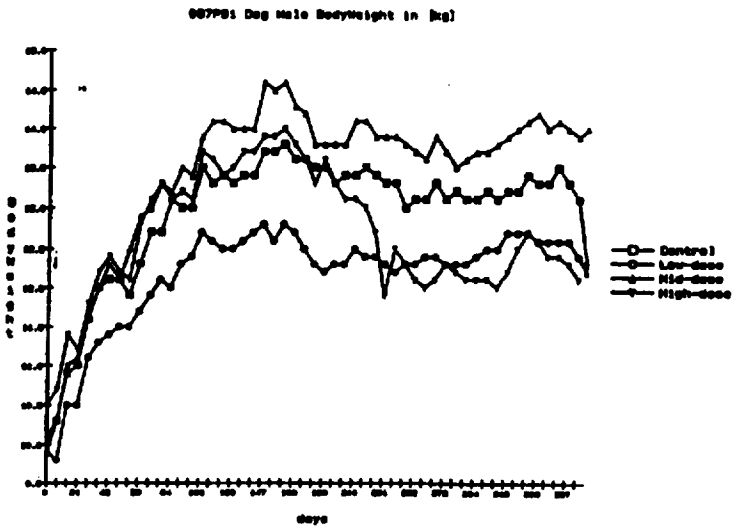
No similar trends were observed in females. No severe individual changes were noted except for moderately decreased erythrocyte parameters and increased platelets in one HDM (#5330). These values recovered after 8 weeks without treatment.

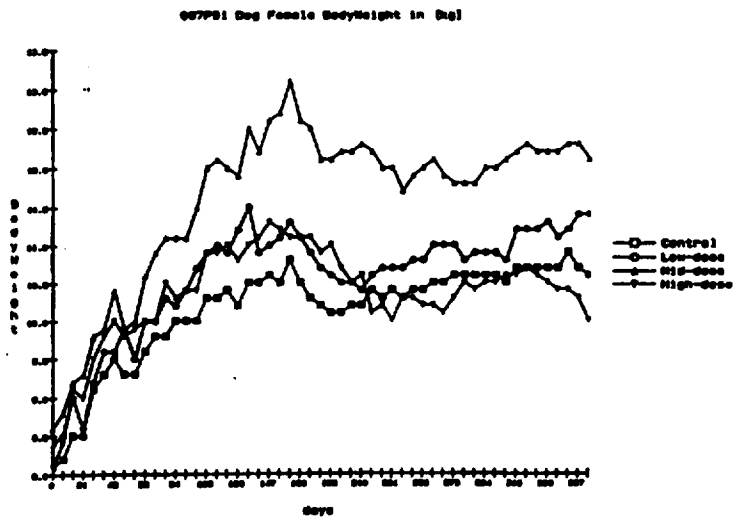
Clinical chemistry: (predose and months 2, 6, 9, and 12; creatinine was not measured)

Some slight but statistically significant variations in cholesterol (decrease), glucose (increase), and potassium (increase) occurred but are not considered biologically relevant. Changes in serum proteins may have resulted from decreased food intake:

↓ total protein	-	HDM (day 178) HDF (day 52, 367)
↓ albumin	-	M, HDM (day 367) M, HDF (day 276, 367)

Fig. 1





↓ gamma globulin	-	HDM (day 178, 367) L, M, HDF (day 178)
↓ A/G ratio	-	HDM (day 178, 276, 367) MDM (day 276, 367)

Increases in bilirubin were evident in MDM and HDM on day 276. The magnitude of the increase was relatively small and probably not toxicologically significant, although mean reductions in red blood cells were noted in the HDM (see above). The sponsor attributed the bilirubin increase to an assay artifact (interference of the assay by tolcapone), but failed to explain how the artefact was limited to one time point and one sex.

Urinalysis: (months 2, 6, and 12)

No treatment-related effect were identified. Small round epithelial cells and protein were found in samples from both control and treated animals.

Organ Weights: (adrenals, brain, heart, kidneys, liver, ovaries, testes)

Statistically significant increases in relative weights of liver (24%) and kidney (22%) occurred in HDM.

Histopathology:

No remarkable treatment-related findings were evident.

Toxicokinetic Analyses: (during weeks 2 and 13, and months 6, 9, and 12)

Increases in exposures to tolcapone were approximately dose-proportional based on AUC, but slightly less than dose proportional based on Cmax. Some drug accumulation was evident when Cmax or AUC determinations were compared among days. Tmax for tolcapone was generally between 1-2 hrs, but was 4.5 hr on days 1 and 15 following the highest test dose . No sex differences in toxicokinetics of tolcapone were evident.

Low levels of the 3-O-methyl metabolite were detected. Among all dosage groups, Cmax ranged from 0.3-1.0 μg/ml, and AUC_{0-8} or $_{0-10}$ ranged from 1.5-6.0 μg.hr/ml. Exposures to the metabolite decreased with increasing dose.

Tolcapone Toxicokinetics in One-Year Dog Study

Cmax (µg/ml)

Day	10 mg/kg	40 mg/kg	150/180 mg/kg
1	8.8	29.0	47.0
15	9.2	32.0	42.4
92	14.1	36.0	53.1
177	11.7	36.0	55.5
273	8.5	28.3	52.1
366	14.5	44.9	94.2

AUC_{0-} (µg.hr/ml)

Day	10 mg/kg	40 mg/kg	150/180 mg/kg
1	20.8	80	361
15	22.4	102	345
92	34.0	127	433
177	32.3	140	486
273	31.4	159	539
366	39.7	143	655

C.4. Reproductive Toxicology

C.4.a. Segment I in Rats: Fertility and Reproduction

GLP — Research Report: J-146,003 — Sponsor Volume: 42

Conducted by: Dept. of Toxicology and Pathology
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Summary:

Tolcapone was administered by gavage at doses of 0, 30, 100 and 300 mg/kg/day to SD-S1c rats (24/sex/dose). Males were treated for 63 days prior to mating, and sacrificed after mating. Females were treated for 14 days prior to mating. One half of the females were treated through gestation, and sacrificed on day 21 for delivery of pups by Caesarean section. The remaining females littered spontaneously and drug treatment was continued through the weaning period (21 days).

Dose-related decreases in body weight and food intake were evident in males over the course of the study, and in females mainly during the pre-mating period. No clearly dug-related effects on fertility, pup skeletal, visceral and external anomalies, litter data or developmental parameters were identified. Five HD dams died during the weaning period; no cause of death was determined. A slightly increased percentage of early deaths per total litters in MD and HD groups was not statistically significant. The pregnancy rate of F1 females from the HD dams was slightly reduced.

One MD dam had a kidney granuloma at necropsy.

Methods:

Dosages: 0, 30, 100, 300 mg/kg/day (Drug Lot: G PUL 573 090) in 0.5% CMC-Na

The high dose was selected based on results of a 4-week oral toxicity study and a Segment II study in which a high rate of mortality was encountered at doses of 400 and 450 mg/kg/day. The low dose is 2.5 times the expected human dose (200 mg, t.i.d.).

Treatment Schedule:

Males: For 63 days prior to mating, and throughout the mating period until recognition of their fertility.

Females: From 14 days prior to mating, through mating, gestation (day 20 in C-section animals) and lactation.

Route of Administration: oral (gavage)

Species/Number: 34 males and 34 females/group (males: 202-239 g; females: 166-192 g)

17 females were randomized to either the a C-section group or the rearing group. Littered pups (8/litter, culled on day 4) were raised to weaning. 2 M and 2 F pups per litter were randomly selected for F_1 fertility studies.

Reproductive/Developmental Assessments:

Males: Gross pathology. Testes and epididymides from males without confirmed fertility were fixed in 15% formalin and examined microscopically.

Females: number of corpora lutea, number and position of implantations (alive fetuses, implantation sites, placental remnant, macerated fetuses, dead fetuses), fetal weights and sex.

Fetuses: (C-section group) External malformations (all), skeletal (1/2 of subjects; Alizarin red staining) and visceral (1/2 of subjects; Wilson's method) abnormalities.

Pups: (rearing group) Live/dead, body weight (days 0, 4, 7, 14, and 21), sex, gross abnormalities. Litters culled to 8 on day 4.

Functional/maturational parameters (erection of pinnae, incisor eruption, eye opening, etc.) were monitored during lactation. At weaning, pups were tested for development of senses (auditory, equilibrium, vision). After weaning, 1 male and 1 female per litter were tested in an emotional (open field) assay and a learning (water maze) test. An additional 1 male and 1 female per litter were tested for reproductive performance.

All animals were examined for pathological changes at termination.

Results:

F_0 Generation

Mortality: 1 HDM on day 44
1 HDF on day 13
1 MDF on day 14

Only the HDF displayed signs of moribundity prior to death (bloody nose, abnormal breathing, decreased activity, hypothermia, hyposthenia). No clear causes of death were identified, but signs of hemorrhage in heart and/or lungs were evident at autopsy. The only histopathological change was erosion and inflammation of the forestomach mucosa, and parakeratosis of the forestomach epithelium in the HDF.

Clinical Signs:

Salivation and discolored urine at $\geq$ 100 mg/kg/day

Body Weight: (Sponsor Figures 2-5)

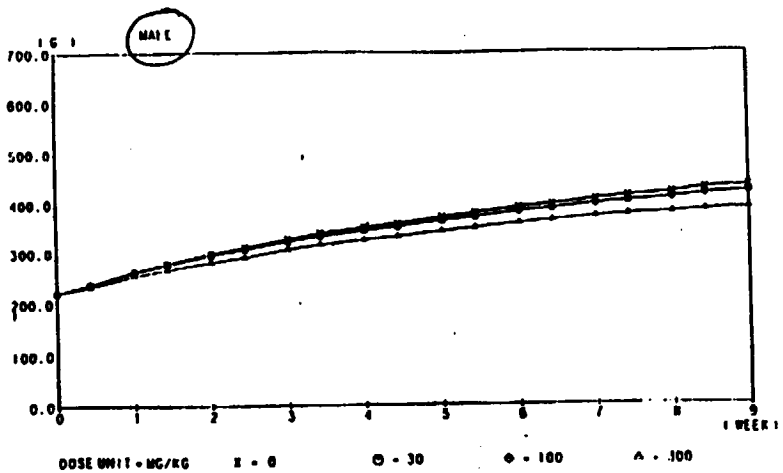


FIG. 3 : REPRODUCTION SEGMENT I STUDY OF RO 40-7592 IN RATS
BODY WEIGHT (BEFORE MATING)

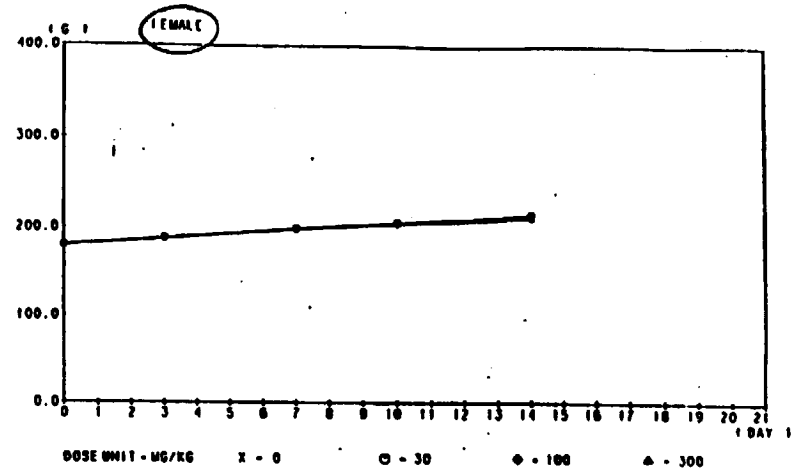


FIG. 5 : REPRODUCTION SEGMENT I STUDY OF RO 40-7592 IN RATS
BODY WEIGHT (BEFORE MATING)

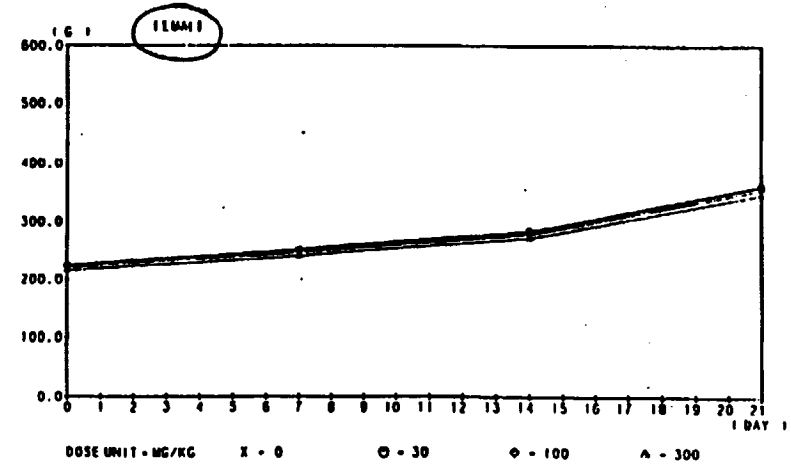


FIG. 4 : REPRODUCTION SEGMENT I STUDY OF RO 40-7592 IN RATS
BODY WEIGHT OF DAMS (PREGNANT)

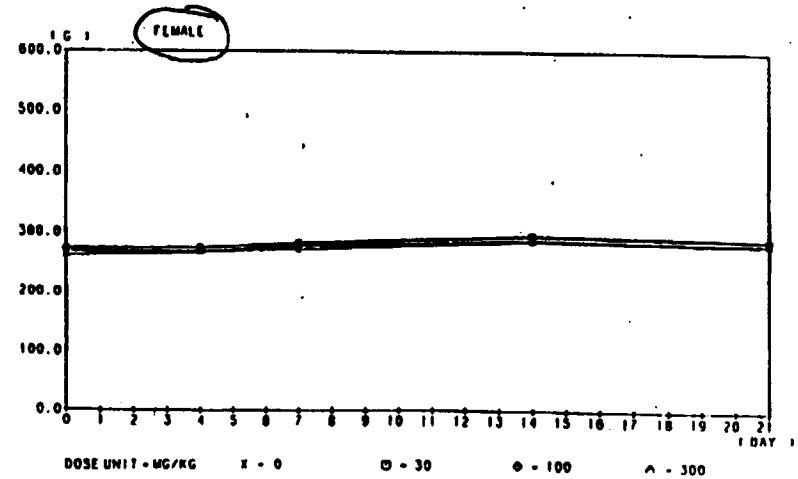


FIG. 6 : REPRODUCTION SEGMENT I STUDY OF RO 40-7592 IN RATS
BODY WEIGHT OF DAMS (LACTATION)

Males: Dose-dependent decreases.
Females: Slight decreases in HDF during the pre-mating period; dose-dependent decreases during the gestation period; no differences during weaning.

Food Intake:

Significantly decreased in the HDM over the course of the study.
Slight decrease in MDF and HDF during pre-mating and gestation.

Reproductive Performance:

All tolcapone-treated animals successfully copulated.

Dam Autopsy:

One MD (C-section group) had a kidney granuloma.

F_1 Generation
C-section group:

Litter data: No clear dose-related effects were evident. A slightly increased percentage of early deaths/total litters was not statistically significant (Sponsor Table 3).

Table 3
Reproduction segment I study of Ro 40-7592 in rats

Item : Litter Data With Caesarean Section - Summary

Control : Ro 40-7592/001 0 mg/kg Carrier : 0.5 % CMC

Dose : (mg/kg)	Control	30	100	300
No of dams	16	17	17	17
Implantations				
Total	207	225	234	223
Mean±SD	12.9±2.5	13.2±3.3	13.8±1.6	13.1±1.1
% to Corpola lutea:mean±SD	96.3±9.7	89.2±20.6	95.6±6.2	98.7±3.0
Alive fetuses				
Male	85	117	96	103
Female	109	95	120	107
Total	194	212	216	210
No. per litters:Mean±SD	12.1±2.7	12.5±3.4	12.7±2.3	12.4±1.5
% to implantations:Mean±SD	93.6±9.4	93.0±9.6	92.0±10.1	94.0±6.1
Early death				
Total	13	12	18	12
% to implantations:Mean±SD	6.4±9.4	6.5±9.8	8.0±10.1	5.8±5.6
Litters with early death	7	7	9	10
% to total litters	43.8	41.2	52.9	58.8
Late death				
Total	0	1	0	1
% to implantations:Mean±SD	0.0±0.0	0.5±1.9	0.0±0.0	0.5±1.9
Litters with late death	0	1	0	1
% to total litters	0.0	5.9	0.0	5.9
Corpola lutea				
Total	215	255	245	226
No. per litters:Mean±SD	13.4±2.2	15.0±2.8	14.4±1.5	13.3±1.0
Body weight of alive fetuses				
Male : Mean±SD	5.1±0.3	5.2±0.3	5.1±0.4	5.2±0.3
Female : Mean±SD	4.8±0.2	4.8±0.3	4.9±0.2	4.9±0.2

Significance of treatment-control difference : * P≤0.05, ** P≤0.01 ; dose response : # P≤0.05, ## P≤0.01

Fetal Examinations:

No external anomalies were identified.
Defects in the heart (1 LD fetus) and vascular rings (1 HD fetus) were identified by visceral examination.
A low incidence of isolated skeletal anomalies and variations were seen in all treatment groups with no suggestion of dose-dependence.

Spontaneous delivery group:

Litter data: Nearly all dams littered on day 22. One of 15 control, 1/17 LD, and 1/16 MD littered on day 23, and 1/16 MD littered on day 21.

Five HD dams died during weaning (on day 0, 1, 2, 4 and 11). No cause of death was determined.

Effects on fetuses and pups:

No treatment-related effects on litter data (number of implantations, live fetuses, etc.), viability or lactation index, or neonatal body weight development.

The only notable difference among treatment groups with respect to developmental parameters was a slight delay in incisor eruption in MD fetuses (76% fair versus 92-93% in other treatment groups).

Skeletal and visceral examination:

Two of 58 MD fetuses had a shortened 13th rib (none in the other treatment groups).

Developmental tests in F1 generation:

No drug-related impairments in emotional (open-field) behavior, learning (water-maze), or reproductive organ development of were evident

In tests of F_1 reproductive performance, copulation percentage rate (LDF = 73%; 100% in other groups) and the pregnancy rate (HDF = 73%; 93-100% in other groups) appeared reduced, but these were not statistically significant. Histopathological findings in the LD animals that did not successfully copulate showed changes consistent with pregnancy (formation of gravid corpus luteum and stratified columnar epithelium in vagina). In the non-pregnant females, one control and one HD animal had no histopathological change, one MD and one HD had enlarged corpora lutea, and one HD had changes similar to those of the LD females that did not successfully copulate. The low incidence and/or independence of dose for these findings are not consistent with a drug relationship.

No differences in litter parameters were evident among groups.

C.4.b. Segment II in Rats: Embryotoxicity and Teratogenicity

GLP — Research Report #: B-154,951 — Sponsor Volume: 43

Conducted by: F. Hoffmann-La Roche Ltd.
CH-4002 Basel
Switzerland

Summary:

Tolcapone was administered orally at doses of 50, 150, 450 mg/kg/day on days 6-15 of gestation (n = 36 mated females per dose). Doses were selected based on a pilot study in which a high dose of 300 mg/kg/day induced slight maternal toxicity (impairment of body weight gain); thus, it was anticipated that a high dose of 450 mg/kg/day would produce overt maternal toxicity. Eighteen of the HD dams died within the first 3 days of dosing. No adverse clinical signs were observed before death, and no adverse necropsy findings were evident. The remaining HD dams were sacrificed without additional drug treatments. The study was completed for the LD and MD animals, but no noteworthy findings were made. A repeat study was conducted using 300 mg/kg/day as the HD.

C.4.c. Repeated Segment II in Rats: Embryotoxicity and Teratogenicity

GLP — Research Report #: B-153,690 — Sponsor Volume: 44

Conducted by: F. Hoffmann-La Roche Ltd.
CH-4002 Basel
Switzerland

Summary:

Tolcapone was administered orally at doses of 0, 50, 150 and 300 mg/kg/day on days 6-15 of gestation (n = 36 mated females per dose). Dams were either sacrificed on day 20, or allowed to litter spontaneously.

Nine HD dams died between days 11-13 of gestation; no cause of death and no adverse necropsy findings were identified. All 9 dams had implantations, and 4 had resorptions. In the remaining animals, body weight development was impaired at the HD during the treatment period.

No drug-related skeletal, visceral or external anomalies were evident in pups from the C-section groups. The number of pups evaluated was similar among groups. In the rearing groups, two HD dams had complete resorptions of litters with a low number of implantations, and two LD dams had complete resorptions. Aside from the HD dams with complete litter resorptions, the number of implantations per dam did not appear to be affected by tolcapone. An equivocal increase in preimplantation loss was noted in HD C-section animals. Since resorptions did not occur at the MD, it is unclear whether the resorptions at the LD were spontaneous or drug-related. Two MD dams delivered several stillborn (7 and 5); the numbers were considered within historical control by the sponsor (range of medians: 0.6 - 4.2). A third MD dam lost 5 pups during lactation. No impairment of pup developmental parameters was evident. Although a strong dose-relationship was not evident for resorptions, stillborns, preimplantation loss, and pup loss, the cumulative findings, including the HD dams that resorbed litters with reduced implantations, suggest that tolcapone may be embryotoxic and fetotoxic.

Methods:

Dosages: 0, 50, 150, 300 mg/kg/day (Drug Lot: G PUL 493 089) in CMC-Na

The high dose is a reduction from the previous Segment II study in which a high maternal mortality rate occurred at 450 mg/kg/day.

Route of Administration: oral (gavage)
Regimen: once daily on days 6-15 of gestation
Species/Number: Fu-albino outbred rats; 36 mated females per dose (195 - 199 g)

Reproductive/Developmental Assessments:

Litter Data:

C-section: Dams were sacrificed on day 20 of gestation. Gross pathology of kidney, lungs, liver, and uterus; numbers of fetuses (viable and dead), corpora lutea, implantations, and resorptions. Fetuses were weighed and examined for external anomalies. One-half of fetuses were examined for skeletal anomalies, and one-half for soft tissue anomalies.

Rearing: Young were raised to weaning. Body weights were recorded on days 1, 4, 12 and 23 of lactation. At termination, pups were examined macroscopically. Only those with external anomalies were examined for skeletal or soft tissue anomalies.

Dams: Dams were euthanized and examined macroscopically. Uteri were removed and the number of implantations were recorded.

Results:

Mortality:

Nine HD dams died on days 11, 12, and 13. Death was rapid after drug administration, but no cause of death was identified, and no adverse necropsy findings were evident.

Clinical Signs:

Regurgitation in all dose groups. Alopecia and nose encrustations in the MD and HD groups. Some HD dams tended to lie on their side briefly after drug treatment.

Body Weight Gain:

Significantly impaired in the HD group over the course of the treatment period; recovered after cessation of treatment (Sponsor Figures 1-3, Table 7).

FIGURE 1 019R91

ENBRYOTOXICITY/TERATOGENICITY STUDY IN RATS WITH ORAL ADMINISTRATION OF Ro 40-7392/001 FROM DO 6 TO 19. SEGMENT II STUDY.

MEAN MATERNAL BODY WEIGHTS DURING GESTATION

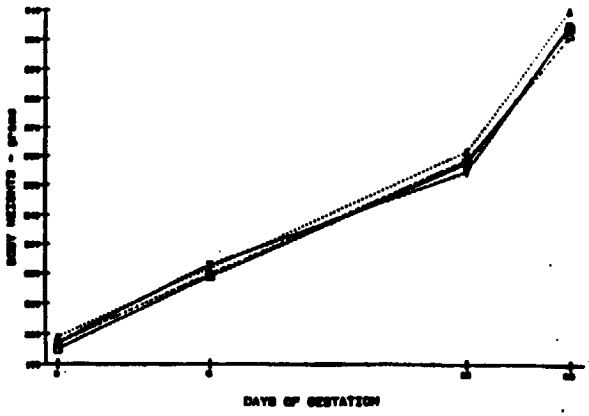


FIGURE 2 019R91

ENBRYOTOXICITY/TERATOGENICITY STUDY IN RATS WITH ORAL ADMINISTRATION OF Ro 40-7392/001 FROM DO 6 TO 19. SEGMENT II STUDY.

MEAN MATERNAL BODY WEIGHTS DURING LACTATION

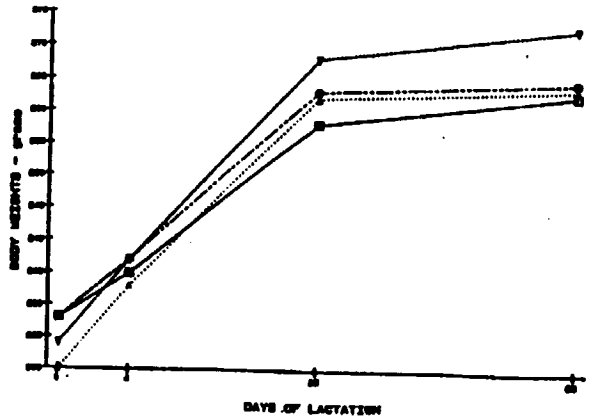
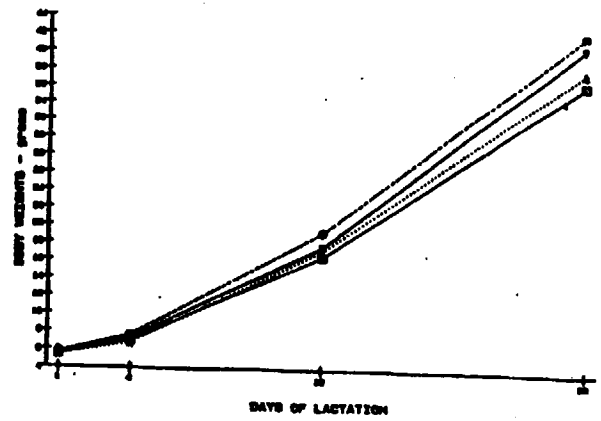


FIGURE 3 019R91

ENBRYOTOXICITY/TERATOGENICITY STUDY IN RATS WITH ORAL ADMINISTRATION OF Ro 40-7392/001 FROM DO 6 TO 19. SEGMENT II STUDY.

MEAN PUP BODY WEIGHTS DURING LACTATION



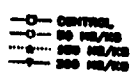


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TABLE 7

ENBRYOTOXICITY AND TERATOGENICITY STUDY IN RATS WITH ORAL ADMINISTRATION OF Ro 40-7392/001. SEGMENT II-STUDY

MEAN MATERNAL BODY WEIGHT CHANGE DURING GESTATION - grams

		CONTROL	50 MG/KG	150 MG/KG	300 MG/KG
DAYS 0 TO 6	MEAN	23.6 d	23.2	23.2	25.0
	S.D.	4.06	4.99	3.62	3.83
	N	35	34	34	25
DAYS 6 TO 16	MEAN	39.3 d	39.4	39.7	33.20
	S.D.	4.62	6.44	4.89	6.50
	N	34	34	34	25
DAYS 16 TO 20	MEAN	46.2 d	41.9	48.5	49.0
	S.D.	6.56	12.77	6.63	6.60
	N	34	34	34	25
DAYS 0 TO 20	MEAN	109.1 d	104.5	111.5	107.0
	S.D.	11.58	16.71	10.11	9.20
	N	34	34	34	25

Statistical key: d=ANOVA + Dunnett-test $ = p<0.001

Necropsy of Dams:

No treatment-related effects were identified. In the HD dams that died or were sacrificed before C-section, 4 had resorptions (1-3 resorptions per dam) and all 9 had implantations.

Litter Data:

Survival/Pregnancy Data (Sponsor Table 1):

819R91

TABLE 1
EMBRYOTOXICITY AND TERATOGENICITY STUDY IN RATS WITH ORAL ADMINISTRATION OF DO 40-7592/001. SEGMENT II-STUDY
SUMMARY OF MATERNAL SURVIVAL AND PREGNANCY STATUS

		CONTROL	50 MG/KG	150 MG/KG	300 MG/KG
Females on study	#	36	36	36	36
C-SECTION GROUP					
Females with evidence of sperm assigned to C-section group	#	21	21	20	29
Nonpregnant,died/sac'd	#	0	0	0	1
Pregnant	#	21	21	20	28
- Died/sac'd during gestation	#	1	0	0	8
Females pregnant and used for analysis at scheduled C-section	#	20	21	20	20
- With total fetal death	#	0	1	0	0
- With viable fetuses	#	20	20	20	20
RAISE GROUP					
Females with evidence of sperm assigned to rearing group	#	15	15	16	7
Nonpregnant	#	1	2	2	0
Pregnant	#	14	13	14	7
- No pups delivered	#	0	0	0	2
Dams delivering	#	14	13	14	5
Dams rearing	#	14	13	14	5
- Complete litterloss	#	0	0	1	0

Two HD dams in the rearing group did not deliver any pups. These animals had a low number of implantations.

Reproduction Data:

A higher number of resorptions were found in the LD group relative to other groups because of a high incidence of resorptions in two litters (10 and 11/litter). Preimplantation losses appeared elevated in the HD C-section group, but this was not indicated as statistically significant (Sponsor Table 9).

819R91

TABLE 9
EMBRYOTOXICITY AND TERATOGENICITY STUDY IN RATS WITH ORAL ADMINISTRATION OF RO 60-7992/001. SEGMENT II-STUDY
SUMMARY OF REPRODUCTION DATA (C-SECTION)

		CONTROL	50 MG/KG	150 MG/KG	300 MG/KG
Pregnant, used for calculation	N	20	21	20	20
Corpora Lutea	N	295	325	301	316
No. per animal	MEAN	14.8	15.5	15.1	15.8
	S.D.	1.97	2.06	2.14	2.40
Preimplantation Loss	N	32	38	25	58
% per group	%	10.8	11.7	8.3	18.4
% per animal	MEAN	10.2	11.9	7.8	17.6
	S.D.	9.31	15.54	10.65	13.79
Implantation Sites	N	263	287	276	258
No. per animal	MEAN	13.1	13.7	13.8	12.9
	S.D.	1.53	2.63	1.88	2.14
Fetuses	N	245	266	252	242
No. per animal	MEAN	12.3	11.6	12.6	12.1
	S.D.	1.68	4.06	1.90	2.31
Alive	%	100.0	100.0	100.0	100.0
Dead	%	0.0	0.0	0.0	0.0
Live Fetuses	N	245	266	252	242
No. per animal	MEAN	12.3	11.6	12.6	12.1
	S.D.	1.68	4.06	1.90	2.31
Dead Fetuses	N	0	0	0	0
No. per animal	MEAN	0.0	0.0	0.0	0.0
	S.D.	0.00	0.00	0.00	0.00
% of impl. per group	%	0.0	0.0	0.0	0.0
% of impl. per animal	MEAN	0.0	0.0	0.0	0.0
	S.D.	0.00	0.00	0.00	0.00

		CONTROL	50 MG/KG	150 MG/KG	300 MG/KG
Pregnant, used for calculation	N	20	21	20	20
Resorptions: Total	N	18	43	24	16
No. per animal	MEAN	0.9	2.0	1.2	0.8
	S.D.	0.97	3.07	1.28	1.24
% of impl. per group	%	6.8	15.0	8.7	6.2
% of impl. per animal	MEAN	6.8	16.4	8.6	5.8
	S.D.	7.76	25.05	8.53	9.09
Resorptions: Early	N	18	43	24	16
% of resorp. per group	%	100.0	100.0	100.0	100.0
Resorptions: Late	N	0	0	0	0
% of resorp. per group	%	0.0	0.0	0.0	0.0
Postimplantation Loss	N	18	43	24	16
No. per animal	MEAN	0.9	2.0	1.2	0.8
	S.D.	0.97	3.07	1.28	1.24
% of impl. per group	%	6.8	15.0	8.7	6.2
% impl. per animal	MEAN	6.8	16.4	8.6	5.8
	S.D.	7.76	25.05	8.53	9.09
Viable Male Fetuses	N	113 f	116	130	129
	%	46.1	47.5	51.6	53.3
Female Fetuses	N	133 f	128	122	113
	%	53.9	52.5	48.4	46.7
Fetal Body Weight (g)	MEAN	3.4	3.4	3.4	3.5
	S.D.	0.16	0.15	0.16	0.15
	N LITTERS	20	20	20	20

Statistical key: f=Chi-square + Fishers exact test

Teratogenicity Data:
C-section Group

External Observations:

No treatment-related anomalies were identified. One control fetus had ectopy of intestines. One HD fetus exhibited exencephaly and spina bifida. Three fetuses from another HD litter exhibited brachygnathia, protruding tongue, and shortened limbs.

Skeletal observations (Alizarin red staining):

The incidence of skeletal variations, retardations and abnormalities was low and unrelated to drug. Specifically, the following were identified:

3 Control	-	sternal abnormalities
1 LD	-	misshapen neural arch
1 LD	-	misaligned sternal element
1 MD	-	asymmetric sternal element
1 HD	-	misaligned ribs
1 HD	-	misshapen vertebral arch
1 HD	-	fused sternal elements
1 HD	-	shortened facial bones

Soft tissue Examination:

No abnormalities were considered related to treatment, although hydroureter was identified in 2 MD and 2 HD fetuses:

1 Control	-	accessory artery between left carotid and subclavian arteries
1 MD	-	missing kidney, hydroureter, enlarged adrenals, and displaced testicle
1 MD	-	cystic kidney, renal hypoplasia, hydronephrosis, and hydroureter
1 HD	-	closed nostrils
2 HD	-	accessory artery between left carotid and subclavian arteries
1 HD	-	anophthalmia, exencephaly, and hydroureter
1 HD	-	hydroureter

Rearing Group

Pregnancy and Litter Data (Sponsor Table 13):

Two HD dams had complete resorptions, but both litters had low numbers of implantations (3 and 5 per litter). The number of liveborn to MD dams was higher than in other groups, as were the number of stillborn (7 in one litter, 5 in a second litter). A third MD dam lost 5 pups during lactation.

The sponsor contends that a litter loss of 5 pups is within historical control range (range of medians: 0.6 - 4.2). The significance of the litter with 7 stillborn pups was discounted as an occurrence "which is sometimes observed in controls also". The remainder of the pups in this litter died during weaning. Because of the low number of HD females that delivered liveborn (5) it is difficult to assess the dose relationship for this parameter. However, the combined findings of a relatively high incidence of MD litters with stillborn (2/14 = 14%) and HD litters with total resorptions (2/7 = 29%) suggests that tolcapone has embryotoxic or fetotoxic potential at doses of 150 and 300 mg/kg/day.

No impairment of pup body weight development was evident in drug-treated animals. The sex ratio in HD litters tended to favor females, but this was not indicated as statistically significant.

Anomalies were seen only in LD fetuses (one hydrocephaly, one kinked tail).

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TABLE 13
EMBRYOTOXICITY AND TERATOGENICITY STUDY IN RATS WITH ORAL ADMINISTRATION OF RO 40-7592/001. SECOND II-STUDY
PREGNANCY AND LITTER DATA (REARING)

		CONTROL	50 MG/KG	150 MG/KG	300 MG/KG
Females on Study	#	15	15	16	7
Females Mated	#	15 f	15	16	7
Mating Index	%	100.0	100.0	100.0	100.0
Females Pregnant	#	14 f	13	14	7
Female Fertility Index	%	93.3	86.7	87.5	100.0
Females with Liveborn	#	14 f	13	14	5
Gestation Index	%	100.0	100.0	100.0	71.4
Females Surviving Delivery	#	14 f	13	14	5
	%	93.3	86.7	87.5	71.4
Duration of Gestation	MEAN	22.2 d	22.2	22.3	22.2
	S.D.	0.58	0.44	0.47	0.45
with Stillborn Pups	#	1 f	0	2	0
	%	7.1	0.0	14.3	0.0
with all Stillborn	#	0 f	0	0	0
	%	0.0	0.0	0.0	0.0
Females with all Resorptions	#	0 f	0	0	2
	%	0.0	0.0	0.0	28.6
Females Pregnant surviving assumed delivery date	#	14 f	13	14	7
	%	93.3	86.7	87.5	100.0
Pups Delivered (total)	#	163	132	188	60
	MEAN	11.6 d	10.2	13.4	12.0
	S.D.	2.95	3.69	1.91	1.87
Liveborn	#	162 f	132	176**	60
Live Birth Index	%	99.4	100.0	93.6	100.0
Stillborn	#	1 f	0	12**	0
	%	0.6	0.0	6.4	0.0

Statistical key: d-ANOVA + Dunnett-test f-Chi-square + Fishers exact test ** = p<0.01

TABLE 13 (cont.)
EMBRYOTOXICITY AND TERATOGENICITY STUDY IN RATS WITH ORAL ADMINISTRATION OF BO 60-7593/001. SEGMENT II-STUDY
PREGNANCY AND LITTER DATA (REARING)

		CONTROL	50 MG/KG	150 MG/KG	300 MG/KG
Females with Entire Liveborn Litter Dying and/or Missing, Cannibalized Sacrificed Moribund					
days 1-4	N	0 f	0	0	0
	%	0.0	0.0	0.0	0.0
days 1-23	N	0 f	0	1	0
	%	0.0	0.0	7.1	0.0
Pups Dying, Missing, Cannibalized, Sacrificed Moribund					
day 1	N	0 f	0	0	0
	%	0.0	0.0	0.0	0.0
days 2-4	N	9 f	2	8	0
	%	5.6	1.5	4.9	0.0
days 5-12	N	2 f	1	11*	3
	%	1.2	0.8	6.3	5.0
days 13-23	N	1 f	0	2	0
	%	0.6	0.0	1.1	0.0
days 1-23	N	12 f	3	21	3
	%	7.4	2.3	11.9	5.0
Pups Surviving 4 days Viability Index	N	153 f	130	160	60
	%	94.4	98.5	93.5	100.0
Pups Surviving 21 days Lactation Index	N	150 f	129	155	57
	%	92.6	97.7	88.1	95.0
Implantation Sites per Litter	N	181	151	200	64
	MEAN	12.9 d	11.6	14.3	12.8
	S.D.	1.38	3.36	1.73	2.17
Resorptions	N	18 f	19	12	4
	%	9.9	12.6	6.0	6.3

Statistical key: d-ANOVA + Dunnett-test f-Chi-square + Fishers exact test * = p<0.05
Resorptions = difference between the number of implantation sites and the number of pups delivered

		CONTROL	50 MG/KG	150 MG/KG	300 MG/KG
Live Pups/Litter					
day 1	MEAN	11.6 d	10.2	12.6	12.0
	S.D.	2.95	3.69	3.16	1.87
	N	14	13	14	5
day 4	MEAN	10.9 d	10.0	12.0	12.0
	S.D.	3.12	3.51	3.49	1.87
	N	14	13	14	5
day 12	MEAN	10.8 d	9.9	11.2	11.4
	S.D.	3.17	3.45	3.88	2.19
	N	14	13	14	5
day 23	MEAN	10.7 d	9.9	11.1	11.4
	S.D.	3.20	3.45	3.87	2.19
	N	14	13	14	5
Pup Weight/Litter (grams)					
day 1	MEAN	5.8 d	5.7	5.4	5.6
	S.D.	0.54	0.51	0.42	0.20
day 4	MEAN	7.0 d	7.6	6.7	7.3
	S.D.	1.15	1.08	1.14	0.75
day 12	MEAN	16.5 d	18.3	17.2	17.6
	S.D.	3.47	2.16	2.76	1.76
day 23	MEAN	36.5 d	43.3*	38.1	40.8
	S.D.	5.89	4.11	5.76	4.63
Sex Ratio - day 23					
Males	N	76 f	71	66	21
	%	50.7	55.8	41.6	36.8
Females	N	74 f	58	90	36
	%	49.3	45.0	58.4	63.2

Statistical key: d-ANOVA + Dunnett-test f-Chi-square + Fishers exact test * = p<0.05

TABLE 13 (cont.)
SUBTOXICITY AND TERATOGENICITY STUDY IN RATS WITH ORAL ; ADMINISTRATION OF BO 49-7393/001. SECOND II-STUDY
PREGNANCY AND LITTER DATA (REARING)

		CONTROL	50 MG/KG	150 MG/KG	300 MG/KG
Females dying or sacrificed moribund postpartum					
day 1	N	0 f	0	0	0
	%	0.0	0.0	0.0	0.0
days 2-4	N	0 f	0	0	0
	%	0.0	0.0	0.0	0.0
days 5-12	N	0 f	0	0	0
	%	0.0	0.0	0.0	0.0
days 13-21	N	0 f	0	0	0
	%	0.0	0.0	0.0	0.0
days 1-21	N	0 f	0	0	0
	%	0.0	0.0	0.0	0.0

Statistical key: f-Chi-square + Fishers exact test

C.4.d. Segment II in Rabbits: Reproduction and Teratogenicity

GLP — Research Report #: B-154,975 — Sponsor Volume: 43

Conducted by: F. Hoffmann- La Roche, ltd.
CH-4002 Basel
Switzerland

Summary:

Tolcapone was administered by gavage at doses of 0, 25, 100 and 400 mg/kg/day to pregnant Swiss hare rabbits (18/dose) from day 6 to 18 of gestation. Fetuses were delivered by Caesarean section on day 29, and maintained in an incubator for 24 hrs. After sacrifice, the offspring were examined for skeletal and visceral abnormalities.

The main drug-related finding was induction of abortions between days 20 and 29 in 2 MD and 6 HD does. One HD doe died on day 22; cause of death was not determined. The median body weight of HD does was markedly lower on day 19 of gestation, but recovered after cessation of treatment. No drug-related external, skeletal or visceral anomalies were identified. Findings from the HD group must be considered equivocal because of the low number of evaluable pups.

Methods:

Dosages: 0, 25, 100, 400 mg/kg/day (Drug Lot: G PUL 573 090) in SSV (0.5% CMC-Na, 0.9% NaCl, 0.5% benzyl alcohol, 0.4% Tween 80, distilled water).

Doses were identical to those used in a pilot study in which a reduction in body weight and post-implantation loss occurred at the mid and high doses (study not submitted to NDA).

Treatment Regimen: Day 6 to day 18 (inclusive) of gestation
Route of Administration: oral (gavage)
Species/Number: 18 outbred Swiss hare rabbits (3177 - 3343 g

Reproductive Parameters Assessed:

Does were sacrificed on day 29 of gestation. Kidneys, liver, and lungs were examined macroscopically. Uteri were removed and examined for the number of corpora lutea, fetal viability/loss, and implantations and resorptions. Viable newborns were maintained in an incubator for 24 hrs, at which point they were weighed, examined for malformations, visceral (gross) and skeletal variations (X-ray), and necropsied. Brains were fixed in formalin/acetic acid and serial-sectioned.

Results:

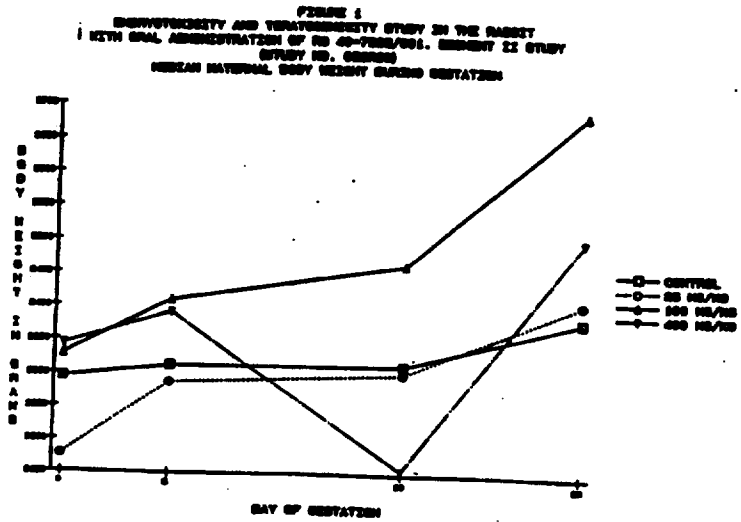
Effects on Does

Mortality: One HD doe died on day 22 of gestation. No cause of death was identified. One control female died from a gavage error.

Clinical Signs: No overt clinical signs of toxicity were noted.

Body Weight Gain:

HD animals experienced a large decrease in median body weights during the dosage period. Body weights recovered thereafter (Sponsor Figure 1):



Necropsy: Two HD does had blood in urine.

Reproductive Parameters:

The number of pregnant females, corpora lutea, and implantation sites were comparable in all groups. Two MD and 6 HD does aborted between days 20-29 (Sponsor Table 5).

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TABLE 5
ENBRYOTOXICITY AND TERATOGENICITY STUDY IN RABBITS WITH ORAL ADMINISTRATION OF RO 40-7592/001. SEGMENT II -. STUDY
SUMMARY OF MATERNAL SURVIVAL AND PREGNANCY STATUS

		CONTROL	25 MG/KG	100 MG/KG	400 MG/KG
Females with evidence of sperm	N	18	18	18	18
Nonpregnant	N	1	1	0	1
Pregnant	N	17	17	18	17
- Died/sac'd during gestation	N	1	0	0	1
- Aborted	N	0	0	2	6
Total no. of females died/ sacrificed moribund	N	1 f	0	0	1
	%	5.6	0.0	0.0	5.6
Females pregnant and used for analysis at scheduled c-section	N	16	17	16	10
- With viable fetuses	N	16 f	17	16	10
	%	100.0	100.0	100.0	100.0

Statistical key: f=Chi-square + Fishers exact test

No treatment-related effects were evident in the number of corpora lutea, preimplantation loss, implantation sites, live/dead fetuses, sex distribution, survivability, or fetal body weights. The percentage of late resorptions tended to be higher in the treated groups, but was not indicated as significant (Sponsor Table 6). Crown-rump length was lower in HD fetuses. This was not correlated with significantly reduced fetal body weights, although the Q1 value in the HD group appeared to be much lower than in any other groups.

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TABLE 6
EMBRYOTOXICITY AND TERATOGENICITY STUDY IN RABBITS WITH ORAL ADMINISTRATION OF BO 60-7592/001. SEGMENT II - STUDY SUMMARY OF REPRODUCTION DATA

		CONTROL	25 MG/KG	100 MG/KG	400 MG/KG
Pregnant, used for calculation	N	16	17	16	18
Resorptions: Total	N	19	19	21	12
No. per animal	MEDIAN	1.0 d	1.0	1.0	1.0
	Q1	0.0	0.0	0.0	0.0
	Q3	2.0	2.0	2.0	2.0
% of impl. per group	%	15.7	13.0	19.1	15.2
% of impl. per animal	MEDIAN	14.6 u	11.1	11.3	11.8
	Q1	0.0	0.0	0.0	0.0
	Q3	25.0	21.6	29.6	32.1
Resorptions: Early	N	16	15	15	8
% of resorp. per group	%	84.2	78.9	71.4	66.7
Resorptions: Late	N	3	4	6	4
% of resorp. per group	%	15.8	21.1	28.6	33.3
Postimplantation Loss	N	18	19	21	12
No. per animal	MEDIAN	1.0 d	1.0	1.0	1.0
	Q1	0.0	0.0	0.0	0.0
	Q3	2.0	2.0	2.0	2.0
% of impl. per group	%	15.7	13.0	19.1	15.2
% impl. per animal	MEDIAN	14.6 u	11.1	11.3	11.8
	Q1	0.0	0.0	0.0	0.0
	Q3	25.0	21.6	29.6	32.1
Viable Male Fetuses	N	30 f	28	26	31
	%	51.5	53.5	47.5	46.3
Female Fetuses	N	27 f	29	32	36
	%	48.5	46.5	52.5	53.7
Fetal Body Weight (g)	MEDIAN	38.9 d	38.5	43.7	38.8
	Q1	37.4	35.3	39.6	29.7
	Q3	44.4	41.5	46.2	44.7
	N LITTERS	16	17	16	18
Crown-rump length (cm)	MEDIAN	9.6 d	9.7	9.6	9.1*
	Q1	9.4	9.3	8.9	8.8
	Q3	9.8	9.9	10.2	9.4
24 Hour Surviving Fetuses	N	85	122	113	57
	%	87.6	96.1	95.8	85.1
Litters with dead fetuses	N	4 f	4	4	5
	%	25.0	23.5	25.0	50.0

Statistical key: d=ANOVA + Dunnett-test f=Chi-square + Fishers exact test u=Kruskal-Wallis + Mann-Whitney U * = p<0.05

Teratology:

External Observations: No treatment-related anomalies were observed.

Visceral Observations: No treatment-related anomalies were observed.

Skeletal Observations: No treatment-related anomalies were observed. One MD fetus exhibited hemivertebrae and multiple rib abnormalities.

C.4.e. Segment III in Rats: Perinatal and Postnatal Toxicity

GLP | Research Report: B-161,856 | Sponsor Volume: 45

Conducted by: F. Hoffmann-LaRoche Ltd.
CH-4002 Basel
Switzerland

Summary:

Tolcapone was administered by gavage at doses of 0, 40, 100 and 250 mg/kg/day to pregnant female Fu-albino rats (24/dose) from day 15 of gestation to day 22 of lactation. The high dosage was reduced to 150 mg/kg/day after 6-8 days of treatment due to a high rate of maternal mortality; 13 dams died between days 19-22 of gestation.

Body weight development was impaired in HD dams during gestation, but not lactation. Litter size was reduced, and the number of resorptions increased at the HD level, but these changes were not statistically significant. A large loss of HD pups from one litter during lactation resulted in a decreased lactation index. Three HD pups that died had thymus hypoplasia that was not considered treatment-related.

In developmental tests, the performance of HDF pups on one of two learning tests was decreased. A slight (non-significant) decrease HDF pup weights was also evident at end of study. No drug-related abnormalities were apparent in pups sacrificed after weaning. Because of the high maternal mortality during gestation and litter loss during lactation phase, there were a low number of evaluable pups from HD dams.

Thus, the HD of 250 mg/kg was maternally toxic. The variations in pup parameters (decreased litter size, lactation index, body weight development, learning performance) are considered equivocal.

Methods:

Dosages: 0, 40, 100, 250/150 mg/kg/day (Drug Lot: G PUL 573 090) prepared in SSV.

The high dose was selected to be slightly lower than the high dose used in previous Segment I and II studies (300 mg/kg/day) that caused significant maternal toxicity. The low dose is four-fold higher than the approximate expected human dose (10 mg/kg/day).

Because of significant mortality at 250 mg/kg/day, the high dose was reduced to 150 mg/kg/day on day 6-8 of treatment.

Route of Administration: oral (gavage)
Treatment Schedule: Daily from day 15 of gestation to day 22 of lactation.
Species/Number: Fu-Albino rats; 96 inseminated females (176 - 212 g)
n = 24/group

Litter parameters assessed:

Size, live/still births, and gross abnormalities were recorded. The number of implantation sites were determined in non-pregnant females.

Functional (auditory startle, pupil contraction) and maturational (hair growth, incisor eruption, ear and eye opening) tests were conducted on all pups during lactation. Pups that died during the study were autopsied; control and HD pups were examined for soft tissue abnormalities.

At day 23 of lactation, 20 male and 20 female F_1 pups per experimental group were randomly selected for tests of learning and memory (water E-maze) at 45 days of age. The dams and remaining weanlings were necropsied, and half the offspring were examined for visceral abnormalities.

Results:

Effects on Dams

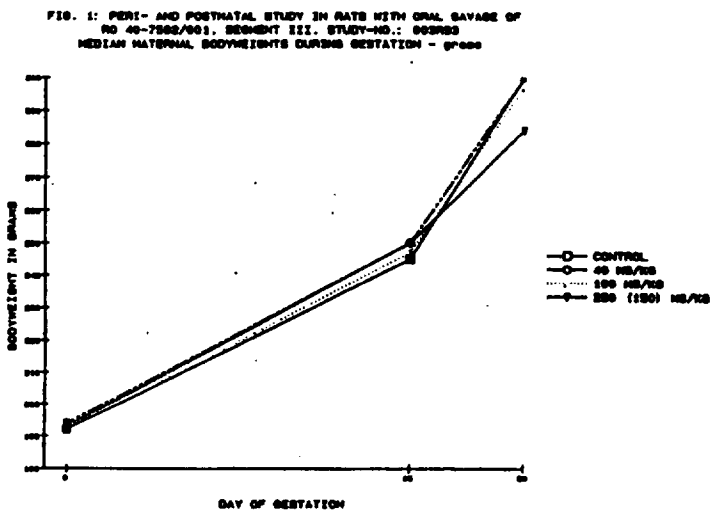
Mortality: 13 HD dams died as a result of drug during gestation (days 19-22). One control, 1 LD, 2 MD and 1 HD dams died from maladministration.

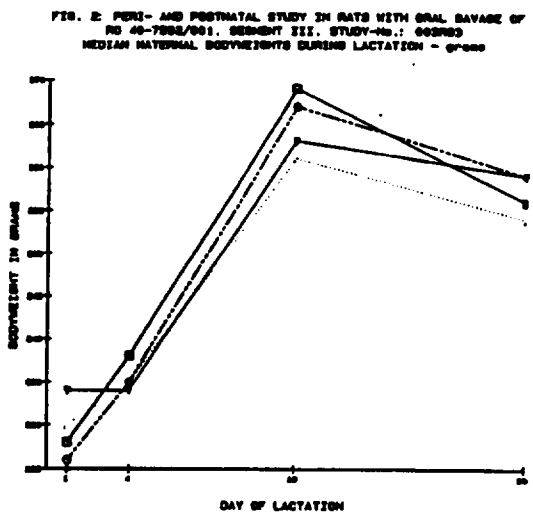
Clinical Signs:

No adverse drug-related clinical signs were observed, including animals that subsequently died.

Body Weight Gain:

Body weight development in HD dams was slightly but significantly impaired during gestation, but no significant difference among groups occurred during lactation (Sponsor Fig. 1&2).





Necropsy: No treatment-related effect was identified.

Gestation: No treatment-related effect on length of gestation.

Litter Parameters

Litter size was reduced and number (percentage) of resorptions increased at the HD level (effects were not statistically significant). Complete litter loss occurred in 1 control, 1 LD and one HD dam.

A large loss of pups during lactation occurred in the control, LD and HD groups. This resulted in a significantly lower "lactation index" (# pups surviving at Lactation day 23/# pups liveborn) in the HD group since a much smaller number of pups were born in this group (Sponsor Table 9).

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TABLE 9
PERI- AND POSTNATAL STUDY IN RATS WITH ORAL GAVAGE OF THE CONT-INHIBITOR BO 40-7892/001. SEGMENT III STUDY.
PREGNANCY AND LITTER DATA (REARING)

		CONTROL	40 MG/KG	100 MG/KG	250 (150) MG/KG
Females on Study	N	24	24	24	24
Females Mated	N	24 f	24	24	24
Mating Index	%	100.0	100.0	100.0	100.0
Females Pregnant	N	22 f	22	19	22
Female Fertility Index	%	91.7	91.7	79.2	91.7
Females with Liveborn	N	21 f	22	17	8‡
Gestation Index	%	95.5	100.0	89.5	36.4
Females Surviving Delivery	N	20 f	21	16	8**
	%	83.3	87.5	66.7	33.3
Duration of Gestation	MEDIAN	22.0 d	22.0	22.0	22.0
	Q1	21.0	22.0	22.0	22.0
	Q3	22.0	22.0	22.0	22.8
with Stillborn Pups	N	2 f	3	3	0
	%	10.0	14.3	18.8	0.0
with all Stillborn	N	0 f	0	0	0
	%	0.0	0.0	0.0	0.0
Females with all Resorptions	N	0 f	0	0	0
	%	0.0	0.0	0.0	0.0
Females Pregnant surviving assumed delivery date	N	20 f	21	16	8**
	%	83.3	87.5	66.7	33.3
Pups Delivered (total)	N	245	251	182	75
	MEDIAN	12.0 d	12.0	12.0	9.5
	Q1	12.0	11.0	10.3	7.3
	Q3	13.0	13.5	13.0	11.0
Liveborn	N	241 f	248	179	75
Live Birth Index	%	98.4	98.8	98.4	100.0
Stillborn	N	4 f	3	3	0
	%	1.6	1.2	1.6	0.0

Statistical key: d=ANOVA + Dunnett-test f=Chi-square + Fishers exact test ** = p<0.01 ‡ = p<0.001

BEST POSSIBLE

TABLE 9 (cont.)
PERI- AND POSTNATAL STUDY IN RATS WITH ORAL GAVAGE OF THE CONT-INHIBITOR BO 40-7592/001. SEGMENT III STUDY.
PREGNANCY AND LITTER DATA (REARING)

		CONTROL	40 MG/KG	100 MG/KG	250 (150) MG/KG
Females with Entire Liveborn Litter Dying and/or Missing, Cannibalized Sacrificed moribund					
days 1-4	N	1 f	0	0	0
	%	4.8	0.0	0.0	0.0
days 1-21	N	1 f	1	0	1
	%	4.8	4.5	0.0	12.5
Pups Dying, Missing, Cannibalized, Sacrificed moribund					
day 1	N	2 f	0	0	2
	%	0.8	0.0	0.0	3.7
days 2-4	N	19 f	11	0	5
	%	7.9	5.2	0.0	6.7
days 5-12	N	1 f	9	1	11
	%	0.4	3.6	0.6	14.7
days 13-21	N	0 f	0	0	1
	%	0.0	0.0	0.0	1.3
days 1-21	N	22 f	22	1	19
	%	9.1	8.9	0.6	25.3
Pups Surviving 4 days Viability Index	N	220 f	235	179	68
	%	91.3	94.8	100.0	90.7
Pups Surviving 21 days Lactation Index	N	219 f	226	178	56
	%	90.9	91.1	99.4	74.7
Implantation Sites per Litter	N	265	275	201	88
	MEDIAN	13.0 d	13.0	13.0	12.0
	Q1	12.0	12.5	11.3	8.0
	Q3	14.0	14.0	14.0	12.8
Resorptions	N	20 f	24	19	13
	%	7.5	8.7	9.5	14.8

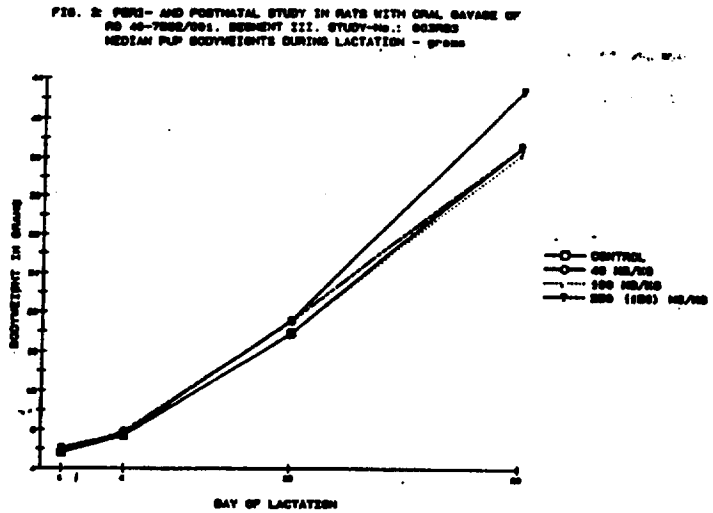
Statistical key: d-ANOVA + Dunnett-test f-Chi-square + Fishers exact test * = p<0.05 ** = p<0.001
Resorptions = difference between the number of implantation sites and the number of pups delivered

		CONTROL	40 MG/KG	100 MG/KG	250 (150) MG/KG
Live Pups/Litter					
day 1	MEDIAN	12.0 d	12.0	12.0	9.5
	Q1	11.0	11.0	10.0	7.3
	Q3	13.0	13.5	13.0	10.8
	N	20	21	16	8
day 4	MEDIAN	12.0 d	12.0	12.0	9.0
	Q1	9.3	10.0	10.0	6.3
	Q3	13.0	13.5	13.0	10.0
	N	20	21	16	8
day 12	MEDIAN	12.0 d	11.0	12.0	7.0
	Q1	9.3	10.0	10.0	3.8
	Q3	13.0	13.0	13.8	10.0
	N	20	21	16	8
day 21	MEDIAN	12.0 d	11.0	12.0	6.5
	Q1	9.3	10.0	10.0	3.8
	Q3	13.0	13.0	13.0	10.0
	N	20	21	16	8
Pup Weight/Litter (grams)					
day 1	MEDIAN	5.6 d	5.7	5.8	6.1
	Q1	5.4	5.4	5.5	5.5
	Q3	6.1	6.0	6.0	6.1
day 4	MEDIAN	7.4 d	7.8	7.5	7.6
	Q1	6.9	6.8	6.8	6.1
	Q3	7.7	8.1	8.1	8.2
day 12	MEDIAN	17.8 d	19.8	17.7	19.1
	Q1	16.4	17.7	16.6	14.1
	Q3	19.3	20.0	19.0	19.9
day 21	MEDIAN	37.2 d	37.2	36.5	42.7
	Q1	33.6	34.9	33.9	34.6
	Q3	39.8	43.5	39.8	45.9
Sex Ratio - day 21					
males	N	119 f	114	86	32
	%	54.3	50.4	48.3	57.1
females	N	100 f	112	92	24
	%	45.7	49.6	51.7	42.9

Statistical key: d-ANOVA + Dunnett-test f-Chi-square + Fishers exact test

Effects on F,

Body weights: No drug-related effects were evident (Sponsor Fig.3).



Physical/Functional Development:

No statistically significant drug-related effects were identified.

Pup Necropsy:

In pups that died during the lactation period, 2 control pups had reduced renal papilla, and 3 HD pups had thymus hypoplasia; LD and MD pups were not examined.

Abnormalities in pups sacrificed after weaning were:

1 control	-	transposition of large vessels
1 "	-	cryptorchidism
1 "	-	renal aplasia
1 LD	-	undescended testes
1 MD	-	renal agenesis
1 MD	-	hydroureter; hydronephrosis
1 MD	-	hydronephrosis
1 HD	-	hydrocephaly

Learning and Memory Performance:

The performance of female pups in learning test 2 was reduced relative to other treatment groups, and is considered a treatment-related effect. Body weight development in HD females also tended to be lower than other treatment groups, but this was not statistically significant (Sponsor Table 17).

TABLE 17
PERI- AND POSTNATAL STUDY IN RATS WITH ORAL GAVAGE OF THE CONT-INHIBITOR RO 40-7592/001. SEGMENT III STUDY.
Statistic of water-maze-test and pup bodyweights

Trials per learning experiment: 6 (1st trial not used for calculation)
Trials per memory experiment : 1

		CONTROL	40 MG/KG	100 MG/KG	250 (150) MG/KG
NUMBER OF ANIMALS PER GROUP	MALES/FEMALES	20 / 20	20 / 20	20 / 20	19 / 20
BODY WEIGHT (MEAN ± SD)	Males	132.9 (±17.15)	141.6 (±15.65)	138.9 (±12.96)	137.4 (±10.43)
	Females	116.1 (±10.63)	119.8 (±12.30)	117.5 (± 9.05)	108.2 (±21.42)
RNING EXPERIMENT 1					
ithmetic mean ± SD of positive trials/animal	Males	4.20 ± 0.83	4.15 ± 0.93	4.05 ± 0.83	4.21 ± 0.92
	Females	4.20 ± 0.83	3.95 ± 1.43	3.85 ± 1.39	3.75 ± 1.37
LEARNING EXPERIMENT 2					
arithmetic mean ± SD of positive trials/animal	Males	3.10 ± 1.59	3.85 ± 1.09	3.30 ± 1.49	3.47 ± 1.58
	Females	3.40 ± 1.35	3.50 ± 1.28	3.50 ± 1.19	2.40 ± 1.70 *
MEMORY EXPERIMENT					
positive / negative	Males	12 / 8	14 / 6	14 / 6	16 / 3
	Females	11 / 9	14 / 6	12 / 8	13 / 7

STATISTICS: Body weights : Jonckheere Test
Learning experiments : Student-T-Test
Memory experiment : Armitage-Trend-Test

* p = < 0.05, ** p = < 0.01,

C.4.f. Combination Tolcapone/Sinemet Segment II Study in Rats: Embryotoxicity and Teratogenicity

GLP Research Report: N-138,697 Sponsor Volumes: 46-48

Conducted by: Developmental Biology Section of Dept. of Toxicology and Pathology
Hoffmann-La Roche, Inc.
Nutley, NJ 07110

Summary:

Tolcapone (10, 30, 50 mg/kg) was orally administered in combination with Sinemet (150 mg/kg) to pregnant rats on days 6-17 of gestation. Tolcapone (50 mg/kg) and Sinemet were also tested individually. Body weight and food intake was significantly reduced in all groups that received Sinemet. No treatment-related effects on reproductive parameters were identified. Average fetal body weights were significantly reduced in all groups that received Sinemet, but not in the group that received tolcapone alone. The combination groups also exhibited possible delays in ossification, but no frank malformations or variations. The effects of the Sinemet combination were attributed to decreased maternal food consumption (skeletal findings only in groups of reduced maternal intake and reduced fetal body weights).

The absence of significant findings in the group treated with 50 mg/kg tolcapone alone suggest that the maternotoxic and fetotoxic effects observed in this study are due to L-DOPA.

Plasma exposures (AUC_{0-24}) to HD tolcapone (alone or in combination with Sinemet) were 1.4-3.6 times higher than exposures in humans receiving the expected maintenance dose of 200 mg, t.i.d. (80 μg.hr/ml). Plasma exposures to L-DOPA (AUC_{0-24}) were 21-40 and 32-110 times higher in Sinemet only and the combination groups, respectively, than human therapeutic exposures (3 μg.hr/ml).

Methods:

Dosages:

Dose Group	Ro 40-7592/001 (mg/kg/day)	Ro 11-7618 (mg/kg/day)	Carbidopa (mg/kg/day)	L-dopa (mg/kg/day)
1	0(Vehicle)	0(Vehicle)	0	0
2	0(Vehicle)	150	30	120
3	10	150	30	120
4	30	150	30	120
5	50	150	30	120
6	50	0(Vehicle)	0	0

The high dose of tolcapone (50 mg/kg/day) was selected on the basis of pilot studies which suggested that higher doses in combination with Sinemet would produce excessive toxicity and deaths. The low dose of tolcapone was selected to approximate the expected human dose.

Route of Administration: oral (gavage)
Regimen: once daily on days 6-17 of gestation
Species/Number: Sprague-Dawley Rats); 87 days old at mating
25 mated females per group

Litter Observations:

All dams were C-sectioned on day 21 of gestation. Thoracic and abdominal organs and viscera were necropsied. Uteri were examined for numbers of implantations, fetuses (viable and dead), and resorptions. Corpora lutea were counted. Fetuses were weighed, sexed and examined for external anomalies. One-half of fetuses were fixed in alcohol and examined for skeletal anomalies using Alizarin Red, and one-half were fixed in Bouin's and examined for visceral anomalies using Wilson's method.

Results:

Effects in Dams

Mortality: One HT/S dam was sacrificed for humane reasons due to self-mutilation. No other maternal mortalities occurred.

Clinical Signs: Self-mutilation was observed in one MT/S and one HT/S in addition to the sacrificed animal. Piloerection was frequently observed in the groups that received Sinemet. Alopecia was also noted as a possible Sinemet-related effect.

Body Weight: Body weight gain was significantly reduced between gestation days 6-11 in all groups that received Sinemet; the reduction in the high-dose tolcapone group was not significant. This led to significantly reduced body weights relative to control in the MT/S and HT/S groups at days 11, 17 and 21 and in the LT/S group at day 11 (Sponsor Table 4).

TABLE 4. SUMMARY OF MATERNAL MEAN BODY WEIGHTS DURING GESTATION

A SEGMENT II TERATOLOGY STUDY OF RO 40-7592/001 AND RO 11-7616 IN THE PREGNANT RAT

EXPERIMENT NUMBER : 08794

DOSE GROUPS : / DOSAGES :	VEHICLE CONTROL 0 MG/KG/DAY	REFERENCE COMP'D 0(VEH.)+150 S.	LOW DOSE 10 T. + 150 S.	MID DOSE 30 T. + 150 S.	HIGH DOSE 60 T. + 150 S.	CONTROL 60 T. + 0(VEH.)
# FEMALES TREATED	25	25	25	25	25	25
# FEMALES PREGNANT (%)	25(100.0)	25(100.0)	25(100.0)	23(92.0)	25(100.0)	22(88.0)
MATERNAL BODY WEIGHT (grams) (MEAN ± S.E.)						
GESTATION DAY 0	275.8 ± 4.2	278.2 ± 2.7	281.8 ± 3.6	272.0 ± 3.1	273.2 ± 3.8	278.0 ± 3.7
GESTATION DAY 6	301.4 ± 5.1	307.3 ± 3.0	305.0 ± 4.7	296.6 ± 3.8	298.7 ± 4.2	302.1 ± 4.0
GESTATION DAY 11	324.6 ± 5.3	319.0 ± 4.0	308.8 ± 4.9**	296.1 ± 5.3***	293.2 ± 4.6***	321.1 ± 4.8
GESTATION DAY 17	370.6 ± 6.8	367.8 ± 4.4	358.5 ± 5.8	344.6 ± 4.8**	341.4 ± 4.7*** [24]	368.3 ± 5.7
GESTATION DAY 21	438.1 ± 7.7	436.8 ± 5.5	423.6 ± 6.3	400.8 ± 5.6**	406.6 ± 5.8** [24]	430.3 ± 7.2

MEAN SIGNIFICANTLY DIFFERENT FROM CONTROLS: * P < 0.05 ** P < 0.01 *** P < 0.001
[ ] NUMBER OF VALUES AVERAGED

Food Consumption: Significantly reduced relative to controls between days 6-11 in all groups that received Sinemet. Some rebound increases in intake were noted at later time points.

Reproduction Parameters:

No treatment-related effects were identified on various reproductive parameters (number of corpora lutea, implantations, preimplantation loss, pregnancy rate, resorption rate, fetal viability). A large number of evaluable pups were present in each dosage group. Average fetal body weights were significantly reduced in all groups that received Sinemet, but not in the tolcapone-only treatment group (Sponsor Table 6).

TABLE 6. CAESAREAN SECTION DATA -- SUMMARY

A SEGMENT II TERATOLOGY STUDY OF RO 40-7592/001 AND RO 11-7618
IN THE PREGNANT RAT

EXPERIMENT NUMBER : 06794

DOSE GROUPS :	VEHICLE CONTROL	REFERENCE COMP'D	LOW DOSE	MID DOSE	HIGH DOSE	CONTROL
DOSAGES :	0 MG/KG/DAY	0(VEH.)+150 S.	10 T. + 150 S.	30 T. + 150 S.	90 T. + 150 S.	90 T. + 0(VEH.)
# Females Mated	25	25	25	25	25	25
# Pregnant (%)	25(100.0)	25(100.0)	25(100.0)	23(92.0)	25(100.0)	22(88.0)
# Pregnancies Aborted	0	0	0	0	0	0
# Premature Births	0	0	0	0	0	0
# Litters with Live Fetuses	25(100.0)	25(100.0)	25(100.0)	23(100.0)	24(96.0)	22(100.0)
# Totally Resorbed Litters	0	0	0	0	0	0
Female Mortality # (%)	0	0	0	0	1(4.0)	0
# Corpora Lutea	392	404	407	356	382	343
Average ± S.E.	15.7 ± 0.5	16.2 ± 0.3	16.3 ± 0.8	15.5 ± 0.4	15.9 ± 0.4	15.6 ± 0.6
# Implantation Sites	388	403	394	352	376	330
Average ± S.E.	15.5 ± 0.5	16.1 ± 0.3	15.8 ± 0.8	15.3 ± 0.4	15.6 ± 0.4	15.0 ± 0.8
Preimplantation Loss (%)	4(1.0)	1(0.2)	13(3.2)	4(1.1)	7(1.8)	13(3.8)
# Viable Fetuses	369	390	367	325	351	314
Average Litter Size ± S.E.	14.8 ± 0.5	15.6 ± 0.3	14.7 ± 0.7	14.1 ± 0.5	14.6 ± 0.4	14.3 ± 0.7
# Dead Fetuses	0	0	0	0	0	0
# Viable Male Fetuses (%)	189(53.9)	181(48.4)	184(52.9)	157(48.3)	170(48.4)	172(54.8)
# Litters with Resorptions (%)	13(52.0)	11(44.0)	14(56.0)	13(56.5)	11(45.8)	10(45.5)
# Resorptions (%)	19(4.9)	13(3.2)	27(6.9)	27(7.7)	24(6.4)	16(4.8)
Average Body Weight (g) of Viable Fetuses ± S.E.	6.44 ± 0.10	6.17 ± 0.09*	4.97 ± 0.09**	4.80 ± 0.10**	4.98 ± 0.10**	6.28 ± 0.10

MEAN SIGNIFICANTLY DIFFERENT FROM CONTROLS: * P < 0.05 ** P < 0.01 *** P < 0.001

Teratogenicity

No treatment-related increases in the incidence of frank malformations (external, skeletal or visceral; Tables 7 & 10) or variations (extra and/or rudimentary ribs; Table 9) were identified. Some group differences in the incidence of retardations were evident, most notably delayed ossification of various structures and wavy ribs in the combination treatment groups (Table 9). Since similar findings were not seen in the tolcapone-only treated animals in this and previous studies, they are probably due to Sinemet. Such findings are considered reversible.

TABLE 7. FETAL EXTERNAL (GROSS) EXAMINATION : FREQUENCY OF VARIOUS FINDINGS (SUMMARY)

A SEGMENT II TERATOLOGY STUDY OF RO 40-7582/001 AND RO 11-7818
IN THE PREGNANT RAT

EXPERIMENT NUMBER : 08794

DOSE GROUPS :	FETUSES 1	2	3	4	5	6	LITTERS 1	2	3	4	5	6
NUMBER EXAMINED LIVE AND DEAD	389	390	387	325	351	314	25	25	25	23	24	22
# LIVE FETUSES	389	390	387	325	351	314						
# DEAD FETUSES	0	0	0	0	0	0						
NUMBER EXAMINED LATE RESORPTIONS	0	0	0	2	2	1	0	0	0	2	2	1
NO REMARKABLE OBSERVATIONS	382	384	384	321	345	314*	25	25	25	23	24	22
I ACCIDENTAL AMPUTATION OF TOES	0	1	0	0	0	0	0	1	0	0	0	0
I SUPERFICIAL/SUBCUTANEOUS HEMATOMA (A SLIGHT HEMORRHAGE)	4	2	1	4	5	0	3	2	1	4	2	0
I AMPUTATION OF LIMB(S) - MECHANICAL DAMAGE	1	1	1	0	1	0	1	1	1	0	1	0
A MENINGOENCEPHALOCELE (PROTRUSION OF MENINGES & BRAIN)	0	0	1	0	0	0	0	0	1	0	0	0
A PROTRUDING TONGUE	0	1	1	0	0	0	0	1	1	0	0	0
A ANASARCA (GENERAL EDEMA - 'SWOLLEN'/'PUFFY' FETUS)	0	1	0	0	0	0	0	1	0	0	0	0
A GASTROSCHISIS (EXPOSURE OF ALL OR MOST ABDOMINAL ORGANS)	0	1	0	0	0	0	0	1	0	0	0	0
I ACCIDENTAL LACERATION	2	1	0	0	0	0	1	1	0	0	0	0
A DEPRESSION ON HEAD	0	1	0	0	0	0	0	1	0	0	0	0
A EXTROMELIA (TOTAL OR PARTIAL ABSENCE OF A LIMB)	0	1	0	0	0	0	0	1	0	0	0	0
A MICROMELIA (SMALL LIMBS)	0	1	0	0	0	0	0	1	0	0	0	0

SIGNIFICANTLY DIFFERENT FROM CONTROLS: * = P < 0.05 ** = P < 0.01
ANOMALY CODE : A = Abnormality (malformation), I = Incidental, R = Retardation, V = Variation
CODE FOR DOSE GROUPS :

1	2	3	4	5	6
0 MG/KG/DAY VEHICLE CONTROL	0(VEH.)+150 S. REFERENCE COMP'D	10 T. + 150 S. LOW DOSE	30 T. + 150 S. MID DOSE	50 T. + 150 S. HIGH DOSE	50 T. + 0(VEH.) CONTROL

TABLE 10. SUMMARY INCIDENCE OF LITTERS AND FETUSES WITH FRANK MALFORMATIONS

A SEGMENT II TERATOLOGY STUDY OF RO 40-7582/001 AND RO 11-7818
IN THE PREGNANT RAT

EXPERIMENT NUMBER : 08794

DOSE GROUPS : / DOSAGES :	VEHICLE CONTROL 0 MG/KG/DAY	REFERENCE COMP'D 0(VEH.)+150 S.	LOW DOSE 10 T. + 150 S.	MID DOSE 30 T. + 150 S.	HIGH DOSE 50 T. + 150 S.	CONTROL 50 T. + 0(VEH.)
# EVALUABLE LITTERS	25	25	25	23	24	22
# EVALUABLE FETUSES	389	390	387	327	353	315
LITTERS AND FETUSES WITH ANY MALFORMATION(S)						
# LITTERS EVALUATED	25	25	25	23	24	22
# LITTERS AFFECTED N(%)	2 (8.0)	4 (16.0)	6 (24.0)	0	2 (8.3)	1 (4.5)
# FETUSES EVALUATED	389	390	387	327	353	315
# FETUSES AFFECTED N(%)	2 (0.5)	4 (1.0)	8 (2.2)	0	2 (0.6)	1 (0.3)
LITTERS AND FETUSES WITH GROSS MALFORMATION(S)						
# LITTERS EVALUATED	25	25	25	23	24	22
# LITTERS AFFECTED N(%)	0	1 (4.0)	1 (4.0)	0	0	0
# FETUSES EVALUATED	389	390	387	327	353	315
# FETUSES AFFECTED N(%)	0	1 (0.3)	1 (0.3)	0	0	0
LITTERS AND FETUSES WITH VISCERAL MALFORMATION(S)						
# LITTERS EVALUATED	25	25	25	23	24	22
# LITTERS AFFECTED N(%)	1 (4.0)	2 (8.0)	2 (8.0)	0	1 (4.2)	1 (4.5)
# FETUSES EVALUATED	177	180	179	158	170	161
# FETUSES AFFECTED N(%)	1 (0.6)	2 (1.1)	3 (1.7)	0	1 (0.6)	1 (0.7)
LITTERS AND FETUSES WITH SKELETAL MALFORMATION(S)						
# LITTERS EVALUATED	25	25	25	23	24	22
# LITTERS AFFECTED N(%)	1 (4.0)	2 (8.0)	4 (16.0)	0	1 (4.2)	0
# FETUSES EVALUATED	192	200	188	166	181	153
# FETUSES AFFECTED N(%)	1 (0.5)	2 (1.0)	5 (2.7)	0	1 (0.6)	0

ONLY FINDINGS CLASSIFIED AS ABNORMALITIES (frank malformations and malpositioning) ARE INCLUDED IN THIS TABLE
SIGNIFICANTLY DIFFERENT FROM CONTROLS: * P < 0.05 ** P < 0.01 *** P < 0.001

TABLE 8. SKELETAL EXAMINATION OF FETUSES : SUMMARY OF OBSERVATIONS

A SEGMENT II TERATOLOGY STUDY OF RO 40-7692/001 AND RO 11-7818
IN THE PREGNANT RAT

EXPERIMENT NUMBER : 06784

DOSE GROUPS :	FETUSES 1	2	3	4	5	6	LITTERS 1	2	3	4	5	6
NUMBER EXAMINED	192	200	186	186	181	183	25	25	26	23	24	22
## FORE.DISTAL												
R ONE DISTAL PHALANX (UPPER LIMB) NOT OSSIFIED	2	3	8	11	7	4	2	2	3	4	4	2
R TWO OR THREE DISTAL PHALANGES (UPPER LIMB) NOT OSSIFIED	0	4	1	3	0	0	0	2	1	3	0	0
I DISTAL PHALANGES (UPPER LIMB) MECHANICAL DAMAGE	1	0	0	0	0	0	1	0	0	0	0	0
R FOUR OR FIVE DISTAL PHALANGES (UPPER LIMB) NOT OSSIFIED	0	0	1	0	0	0	0	0	1	0	0	0
## SPINAL COLUMN												
I THE FETUS ACCIDENTALLY AMPUTATED IN THE LUMBAR REGION	2	2	0	0	1	1	2	2	0	0	1	1
## LARYNX												
R HYOID BODY UNOSSIFIED	0	0	0	1	2	0	0	0	0	1	1	0
R ODONTOID PROCESS BIPARTITE	0	1	2	0	2	0	0	1	1	0	2	0
## HINDLIMBS												
I HINDLIMB(S) DAMAGED DURING PROCESSING	0	1	1	1	0	0	0	1	1	1	0	0
## METATARSALS												
R ONE METATARSAL (LOWER LIMB) NOT OSSIFIED	19	36	53*	61**	37	18	8	13	15*	14*	12	7
## HIND.PROXIMAL												
R FOUR PROXIMAL PHALANGES (LOWER LIMB) NOT OSSIFIED	89	133	149**	128*	148**	78	21	22	25	23	24	19
R TWO OR THREE PROXIMAL PHALANGES (LOWER LIMB) NOT OSSIFIED	33	32	21	22	24	26	16	14	11	13	10	12
R ONE PROXIMAL PHALANX (LOWER LIMB) NOT OSSIFIED	28	19	12*	18	9*	24	15	8	7*	7*	8	13
## HIND.DISTAL												
R ONE DISTAL PHALANX (LOWER LIMB) NOT OSSIFIED	0	2	1	1	0	1	0	1	1	1	0	1
R TWO OR THREE DISTAL PHALANGES (LOWER LIMB) NOT OSSIFIED	0	0	2	5	0	0	0	0	2	2	0	0

SIGNIFICANTLY DIFFERENT FROM CONTROLS * P < 0.05, ** P < 0.01
ANOMALY CODE : A = Abnormality (malformation) I = Incidental R = Retardation V = Variation
CODE FOR DOSE GROUPS :

1	2	3	4	5	6
0 MG/KG/DAY VEHICLE CONTROL	0(VEH.)+150 S. REFERENCE COMP'D	10 T. + 150 S. LOW DOSE	30 T. + 150 S. MID DOSE	50 T. + 150 S. HIGH DOSE	50 T. + 0(VEH.) CONTROL

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Toxicokinetics:

Plasma samples were obtained from satellite groups of animals on days 6 and 11 of gestation and analyzed for tolcapone, L-DOPA and metabolites. Tolcapone did not influence peak levels of L-DOPA, but increased the AUC for L-DOPA. A dose-related increase in L-DOPA exposure due to tolcapone was not observed, as relatively comparable L-DOPA AUCs were obtained with all three tolcapone doses. L-DOPA tended to reduced peak levels of high dose tolcapone, with only a slight effect on tolcapone AUC. Levels of 3-O-methyl-DOPA were markedly reduced by tolcapone (Sponsor Tables A&C).

Table A : Pharmacokinetic parameters of Ro 40-7592

	Cmax (µg/ml)	
Dosage group	Day 6	Day 11
3	2.72 - 3.20	2.38 - 2.53
4	11.4 - 13.2	6.23 - 9.06
5	11.4 - 14.2	8.99 - 12.6
6	18.8 - 25.6	32.2 - 34.6

	AUC0-8h (h•µg/ml)	
Dosage group	Day 6	Day 11
3	30 - 46	35 - 55
4	100 - 193	52 - 98
5	115 - 179	138 - 198
6	110 - 198	158 - 287

L-DOPA

Table C : Pharmacokinetic parameters of Ro 40-7592

	Cmax (µg/ml)	
Dosage group	Day 6	Day 11
2	9.13 - 16.5	9.91 - 15.2
3	9.28 - 20.2	22.3 - 22.6
4	20.7 - 21.4	23.5 - 42.5
5	17.6 - 19.0	24.9 - 29.1

	AUC0-8h (h•µg/ml)	
Dosage group	Day 6	Day 11
2	67.5 - 113	64.0 - 121
3	94.8 - 204	120 - 270
4	198 - 252	194 - 329
5	185 - 200	192 - 264

Group 2 : 120 mg/kg/day L-DOPA plus 30 mg/kg/day carbidopa

Group 3 : 120 mg/kg/day L-DOPA plus 30 mg/kg/day carbidopa and 10 mg/kg/day Ro 40-7592

Group 4 : 120 mg/kg/day L-DOPA plus 30 mg/kg/day carbidopa and 30 mg/kg/day Ro 40-7592

Group 5 : 120 mg/kg/day L-DOPA plus 30 mg/kg/day carbidopa and 90 mg/kg/day Ro 40-7592

C.4.g. Combination Tolcapone/Sinemet Segment II Study in Rabbits: Embryotoxicity and Teratogenicity

GLP | Research Report: N-138,698 | Sponsor Volumes: 49-50

Conducted by: Developmental Biology Section of Dept. of Toxicology and Pathology
Hoffmann-La Roche, Inc.
Nutley, NJ 07110

Summary:

Tolcapone (25 and 100 mg/kg) was orally administered in combination with Sinemet (100 mg/kg) to pregnant rabbits (n = 20 group) on days 6-18 of gestation. Tolcapone (100 mg/kg) and Sinemet were also tested individually.

Food intake and body weight gain were reduced in all drug treatment groups, except for the tolcapone-only group where the reduction was not significant. No treatment-related effects on reproductive parameters were identified. Average fetal body weights were significantly reduced in the Sinemet-only treatment group. In the HT/S group, slightly (non-significantly) increased incidences of malformed fetuses were observed, mainly in the litters of three does that showed marked maternal toxicity (1 S alone group, 2 HT/S groups). Syndactyly was the most frequently observed malformation (4 HT/S fetuses).

Toxicokinetic analyses showed that the low dose of tolcapone increased L-DOPA AUC by 1.5-2X versus the Sinemet only treatment group; the higher tolcapone dose did not increase L-DOPA exposures any further. Plasma exposures (AUC_{0-24}) to tolcapone (consideration of all treatment groups) were 0.125-0.5 times the exposure in humans at the projected maintenance dose of 200 mg, t.i.d. (80 μg.hr/ml). Exposures to L-DOPA were 8-11 times (Sinemet only) and 11-22 times (combination groups) the human therapeutic exposure (3 μg.hr/ml).

Methods:

Treatment Groups:

Group	Ro 40-7592/001 (mg/kg/day)	Ro 11-7618 (mg/kg/day)	Carbidopa (mg/kg/day)	L-dopa (mg/kg/day)
1	0 (Vehicle)	0 (Vehicle)	0	0
2	0 (Vehicle)	100	20	80
3	25	100	20	80
4	100	100	20	80
5	100	0 (Vehicle)	0	0

The high dose of tolcapone (100 mg/kg/day) was selected on the basis of pilot studies in which stomping (hyperexcitability) was induced by Sinemet alone and in combination with 100 mg/kg/day tolcapone. No other adverse effects resulted from these treatments. The low dose of tolcapone was selected as a "No Effect" dose.

Route of Administration: oral (gavage)
Regimen: once daily on days 6-18 of gestation; animals were sacrificed on day 29
Species/Number: New Zealand white rabbits (Hazelton); 3.0 - 5.5 kg at mating
20 mated females per group

Litter Observations:

All does were C-sectioned on day 29 of gestation. Thoracic and abdominal organs and viscera were necropsied. Uteri were examined for numbers of implantations, fetuses (viable and dead), and resorptions. Corpora lutea were counted. Fetuses were weighed and examined for external anomalies. All fetuses were examined for visceral abnormalities, sexed, and processed for skeletal examination.

Results:

Effects in Dams

Mortality: One doe from the Sinemet only group was sacrificed following a spontaneous abortion.

Clinical Signs: Stomping (hyperexcitability) was the most prominent sign, and was observed in all groups the received Sinemet alone and in combination with tolcapone.

Body Weight: Body weight gain was significantly reduced between gestation days 18-29 in all groups that received Sinemet, and between days 23-29 in the tolcapone only treatment group (not significant, Sponsor Table 5). Body weights among the drug-treated groups did not significantly differ.

TABLE 5. SUMMARY OF MATERNAL MEAN BODY WEIGHT CHANGES (grams and percent) DURING GESTATION

A SEGMENT II TERATOLOGY STUDY OF Ro 40-7592/001 IN COMBINATION WITH Ro 11-7810 ADMINISTERED ORALLY TO PREGNANT NEW RABBITS

EXPERIMENT NUMBER : 06792

DOSE GROUPS : DOSAGES :	VEHICLE CONTROL 0(VEH) + 0(VEH)	REFERENCE COMP'D 0(VEH) + 100 S.	LOW DOSE 25 T. + 100 S.	HIGH DOSE 100 T. + 100 S.	CONTROL 100 T. + 0(VEH)
# FEMALES TREATED	20	20	20	20	20
# FEMALES PREGNANT (%)	19(95.0)	19(95.0)	20(100.0)	19(95.0)	20(100.0)
MATERNAL BODY WEIGHT CHANGE (grams) (MEAN ± S.E.)					
GESTATION DAYS 0 - 6	230.0 ± 37.2	255.7 ± 18.2	311.1 ± 21.8	290.1 ± 22.0	266.4 ± 18.6
% Change (Mean ± SE)	6.5 ± 1.1	6.9 ± 0.5	8.7 ± 0.7	8.0 ± 0.7	7.6 ± 0.6
GESTATION DAYS 6 - 12	64.9 ± 27.5	70.4 ± 10.6	38.6 ± 15.9	22.8 ± 16.3	12.6 ± 12.6
% Change (Mean ± SE)	1.8 ± 0.8	1.8 ± 0.4	1.1 ± 0.4	0.6 ± 0.5	0.3 ± 0.3
GESTATION DAYS 12 - 18	136.2 ± 21.1	100.2 ± 16.0	150.1 ± 10.3	92.4 ± 20.2	102.5 ± 19.8
% Change (Mean ± SE)	3.4 ± 0.5	2.8 ± 0.4	3.8 ± 0.3	2.4 ± 0.6	2.8 ± 0.4
GESTATION DAYS 6 - 18	201.1 ± 38.9	170.6 ± 27.8	188.8 ± 18.1	115.3 ± 33.1	115.3 ± 23.4
% Change (Mean ± SE)	5.3 ± 1.1	4.8 ± 0.7	4.8 ± 0.5	3.1 ± 0.9	3.0 ± 0.6
GESTATION DAYS 18 - 23	54.8 ± 16.8	2.8 ± 19.2*	1.8 ± 10.1*	-4.6 ± 12.2*	63.3 ± 13.8
% Change (Mean ± SE)	1.4 ± 0.4	0.8 ± 0.5*	0.1 ± 0.2*	-0.1 ± 0.3**	1.8 ± 0.3
GESTATION DAYS 23 - 29	136.0 ± 14.0	98.8 ± 29.1*	120.3 ± 14.5	98.7 ± 18.6*	46.8 ± 30.8*
% Change (Mean ± SE)	3.4 ± 0.4	1.4 ± 0.8*	3.0 ± 0.4	2.2 ± 0.6*	1.3 ± 1.0*
		[16]			
GESTATION DAYS 18 - 29	192.8 ± 23.1	85.1 ± 43.0*	122.2 ± 19.4*	82.2 ± 24.0**	110.2 ± 46.4
% Change (Mean ± SE)	4.8 ± 0.8	1.8 ± 1.1*	3.0 ± 0.5*	2.1 ± 0.6**	2.9 ± 1.1
		[16]			

MEAN SIGNIFICANTLY DIFFERENT FROM CONTROLS: * P < 0.05 ** P < 0.01 *** P < 0.001
[ ] NUMBER OF VALUES AVERAGED

Food Consumption: Food consumption was significantly reduced relative to controls in all drug-treated groups between days 12-29. Two Sinemet-only does, and one HT/S dose were noted to eat noticeably less than other does.

Reproduction Parameters:

No treatment-related effects were identified on various reproductive parameters (number of corpora lutea, implantations, preimplantation loss, pregnancy rate, resorption rate, fetal viability). One Sinemet -only treated doe aborted, and one tolcapone-only treated doe delivered prematurely. However, a large number of evaluable pups were present in each dosage group. Average fetal body weights were significantly reduced in the Sinemet-only treatment group (Sponsor Table 6).

TABLE 6. CAESAREAN SECTION DATA -- SUMMARY

A SEGMENT II TERATOLOGY STUDY OF Ro 40-7592/001 IN COMBINATION WITH Ro 11-7810 ADMINISTERED ORALLY TO PREGNANT NZW RABBITS

EXPERIMENT NUMBER : 96702

DOSE GROUPS : DOSAGES :	VEHICLE CONTROL 0(VEH) + 0(VEH)	REFERENCE COMP'D 0(VEH) + 100 S.	LOW DOSE 25 T. + 100 S.	HIGH DOSE 100 T. + 100 S.	CONTROL 100 T. + 0(VEH)
# Females Mated	20	20	20	20	20
# Pregnant (%)	19(95.0)	19(95.0)	20(100.0)	19(95.0)	20(100.0)
# Pregnancies Aborted	0	1(5.3)	0	0	0
# Premature Births	0	0	0	0	1(5.0)
# Litters with Live Fetuses	19(100.0)	18(94.7)	20(100.0)	18(100.0)	19(95.0)
# Totally Resorbed Litters	0	0	0	0	0
Female Mortality # (%)	0	0	0	0	0
# Corpora Lutea Average ± S.E.	167 8.8 ± 0.4	166 8.8 ± 0.3	178 8.9 ± 0.3	163 8.8 ± 0.4	167 8.3 ± 0.4
# Implantation Sites Average ± S.E.	155 8.2 ± 0.3	144 8.0 ± 0.5	168 8.3 ± 0.5	144 7.8 ± 0.5	148 7.8 ± 0.4
Preimplantation Loss (%)	12(7.2)	14(8.9)	10(5.7)	19(11.7)	8(5.1)
# Viable Fetuses Average Litter Size ± S.E.	148 7.7 ± 0.5	130 7.2 ± 0.5	151 7.8 ± 0.8	134 7.1 ± 0.5	141 7.4 ± 0.4
# Dead Fetuses	0	0	0	0	0
# Viable Male Fetuses (%)	77(52.7)	58(44.6)	74(49.0)	75(56.0)	84(45.4)
# Litters with Resorptions (%)	5(26.3)	8(44.4)	7(35.0)	8(42.1)	6(31.6)
# Resorptions (%)	8(5.8)	14(9.7)	15(8.9)	10(6.9)	8(5.4)
Average Body Weight (g) of Viable Fetuses ± S.E.	43.00 ± 1.22	38.93 ± 1.07[b]	41.10 ± 1.25	40.31 ± 1.38	43.30 ± 1.43

MEAN SIGNIFICANTLY DIFFERENT FROM CONTROLS: * P < 0.05 ** P < 0.01 *** P < 0.001

Teratogenicity

External Examination: Syndactyly was found in 4 HT/S fetuses (Sponsor Table 7).

TABLE 7. FETAL EXTERNAL (GROSS) EXAMINATION : FREQUENCY OF VARIOUS FINDINGS (SUMMARY)

A SEGMENT II TERATOLOGY STUDY OF Re 40-7582/001 IN COMBINATION WITH Re 11-7818 ADMINISTERED ORALLY TO PREGNANT NZW RABBITS

EXPERIMENT NUMBER : 00782

DOSE GROUPS :	FETUSES 1	2	3	4	5	LITTERS 1	2	3	4	5
NUMBER EXAMINED LIVE AND DEAD	148	130	161	134	141	18	18	20	18	19
# LIVE FETUSES	148	130	161	134	141					
# DEAD FETUSES	0	0	0	0	0					
NO REMARKABLE OBSERVATIONS	145	129	161	129	140	18	18	20	18	19
A UMBILICAL HERNIA (PROTRUSION OF A SMALL LOOP OF THE BOWEL)	0	0	0	1	1	0	0	0	1	1
A SYNDACTYLY (ABNORMAL FLESHY OR BONY FUSION OF FINGERS)	0	0	0	4	0	0	0	0	2	0
I SUPERFICIAL/SUBCUTANEOUS HEMATOMA (A SLIGHT HEMORRHAGE)	1	1	0	0	0	1	1	0	0	0
NUMBER EXAMINED LATE RESORPTIONS	1	0	11	3	2	1	4	4	3	2
A GASTROSCHISIS (EXPOSURE OF ALL OR MOST ABDOMINAL ORGANS)	0	1	0	0	0	0	1	0	0	0
A ACEPHALY (ABSENCE OF HEAD)	0	1	0	0	0	0	1	0	0	0
A ANOPHTHALMIA (NO EYES)	0	1	0	0	0	0	1	0	0	0

SIGNIFICANTLY DIFFERENT FROM CONTROLS: * = P < 0.05 ** = P < 0.01
ANOMALY CODE : A = Abnormality (malformation), I = Incidental, R = Retardation, V = Variation
CODE FOR DOSE GROUPS :

1	2	3	4	5
0(VEH) + 0(VEH) VEHICLE CONTROL	0(VEH) + 100 S. REFERENCE COMP'D	25 T. + 100 S. LOW DOSE	100 T. + 100 S. HIGH DOSE	100 T. + 0(VEH) CONTROL

Skeletal Examinations: Delays in ossification of the hind middle phalanges and skull appeared to occur at a higher incidence in the HT/S group, but these were not indicated as significant (Sponsor Table 9).

TABLE 8. SKELETAL EXAMINATION OF FETUSES : SUMMARY OF OBSERVATIONS

EXPERIMENT

A SEGMENT II TERATOLOGY STUDY OF Re 40-7582/001 IN COMBINATION WITH Re 11-7818 ADMINISTERED ORALLY TO PREGNANT NZW RABBITS

DOSE GROUPS :	FETUSES 1	2	3	4	5	LITTERS 1	2	3	4	5
NUMBER EXAMINED	148	130	161	134	141	18	18	20	18	19
LIVE	148	130	161	134	141					
DEAD	0	0	0	0	0					
NO REMARKABLE FINDINGS	37	30	62	35	38	13	11	18	18	13
## SKULL										
I SKULL CAP (CALVARIUM) VERY LOOSELY ATTACHED; MAY FALL OFF	1	2	0	1	0	1	1	0	1	0
I SKULL CAP MISSING - POSSIBLY LOST DURING PROCESSING	0	0	1	0	0	0	0	1	0	0
A ENLARGED FONTANEL (WITH ACCOMPANYING BRAIN ANOMALIES)	0	0	1	0	0	0	0	1	0	0
R FRONTAL-INCOMPLETELY OSSIFIED	0	0	1	0	0	0	0	1	0	0
R PARIETAL-INCOMPLETELY OSSIFIED	0	0	1	2	0	0	0	1	2	0
R ENLARGED FONTANEL	0	1	0	5	0	0	1	0	4	0
R INTERPARIETAL-INCOMPLETELY OSSIFIED	0	0	0	1	0	0	0	0	1	0
R SUPRAOCCIPITAL-INCOMPLETELY OSSIFIED	0	0	0	1	0	0	0	0	1	0
## THORACIC VERT.										
A HEMIVERTEBRA	0	1	1	0	0	0	1	1	0	0
A THORACIC VERTEBRAE MISALIGNED	0	1	0	0	0	0	1	0	0	0
## LUMBAR VERT.										
R TWO OR THREE LUMBAR CENTRA INCOMPLETELY OSSIFIED	0	0	1	0	0	0	0	1	0	0
A LUMBAR VERTEBRAE MISALIGNED	1	0	0	0	0	1	0	0	0	0
## CAUDAL VERT.										
I TAIL (CAUDAL VERTEBRAE) DAMAGED DURING PROCESSING	4	4	7	3	8	4	3	8	2	7
## STERNEBRAE										
R ONE STERNEBRA UNOSSIFIED	10	23	9	19	10	8	8	5	9	8
I STERNEBRA(E) MISSING - MECHANICAL DAMAGE	2	0	1	0	1	2	0	1	0	1
R ONE BIPARTITE STERNEBRA	2	0	0	1	1	2	0	0	1	1
R TWO OR MORE IRREGULARLY-SHAPED STERNEBRAE	1	1	2	0	1	1	1	2	0	1
R STERNEBRAE FUSED	1	2	5	7	4	1	2	4	4	4
R TWO OR MORE STERNEBRAE UNOSSIFIED	3	4	0	5	4	1	3	0	2	2

SIGNIFICANTLY DIFFERENT FROM CONTROLS * P < 0.05, ** P < 0.01
ANOMALY CODE : A = Abnormality (malformation) I = Incidental R = Retardation V = Variation
CODE FOR DOSE GROUPS :

1	2	3	4	5
0(VEH) + 0(VEH) VEHICLE CONTROL	0(VEH) + 100 S. REFERENCE COMP'D	25 T. + 100 S. LOW DOSE	100 T. + 100 S. HIGH DOSE	100 T. + 0(VEH) CONTROL

TABLE 8. SKELETAL EXAMINATION OF FETUSES : SUMMARY OF OBSERVATIONS

A SEGMENT II TERATOLOGY STUDY OF Ro 40-7692/001 IN COMBINATION WITH Ro 11-7618 ADMINISTERED ORALLY TO PREGNANT NZW RABBITS

EXPERIMENT NUMBER : 06792

	FETUSES					LITTERS				
DOSE GROUPS :	1	2	3	4	5	1	2	3	4	5
NUMBER EXAMINED	148	130	151	134	141	19	18	20	19	19
## STERNEBRAE (Continued)										
R ONE IRREGULARLY-SHAPED STERNEBRA	0	1	1	1	1	0	1	1	1	1
R ONE OR MORE STERNEBRAE SPLIT (CLEAVED)	0	0	1	0	1	0	0	1	0	1
A MISALIGNED (SCRAMBLED) STERNEBRAE	0	1	0	0	0	0	1	0	0	0
## RIBS										
V RIB 13TH, BILATERAL	60	29	29*	21*	40	15	12	8*	8*	14
V RIB 13TH, RUDIMENTARY, UNILATERAL	21	11	22	18	21	11	8	13	13	12
V RIB 13TH, UNILATERAL	10	8	8	8	8	7	6	6	4	6
I RIB(S) - MECHANICAL DAMAGE	5	5	10	7	6	3	3	8	4	4
V RIB 13TH, RUDIMENTARY, BILATERAL	10	18	15	15	13	7	8	8	10	8
A RIB BIFURCATED	0	0	1	0	0	0	0	1	0	0
A TWO RIBS ORIGINATING FROM SAME VERTEBRA	0	1	0	0	0	0	1	0	0	0
## FORELIMBS										
I FORELIMB(S) DAMAGED DURING PROCESSING	0	0	1	1	0	0	0	1	1	0
## METACARPALS										
R ONE METACARPAL (FORELIMB) NOT OSSIFIED	0	4	2	3	4	0	3	2	2	3
## FORE.PROXIMAL										
I PROXIMAL PHALANGES (FOREPAWS) MISSING - MECHANICAL DAMAGE	0	0	0	1	0	0	0	0	1	0
## FORE.MIDDLE										
R ONE MIDDLE PHALANX (FOREPAWS) NOT OSSIFIED	15	39*	29	30	17	8	13	8	8	8
I MIDDLE PHALANGES MISSING (FOREPAWS) - MECHANICAL DAMAGE	0	3	0	1	1	0	3	0	1	1
## FORE.DISTAL										
I DISTAL PHALANGES MISSING (FOREPAWS) - MECHANICAL DAMAGE	0	3	0	1	1	0	3	0	1	1

	FETUSES					LITTERS				
DOSE GROUPS :	1	2	3	4	5	1	2	3	4	5
NUMBER EXAMINED	148	130	151	134	141	19	18	20	19	19
## LARYNX										
R HYOID BODY BIPARTITE	4	4	0	2	2	3	3	0	2	2
R HYOID BODY INCOMPLETELY OSSIFIED	0	4	5	1	4	0	3	3	1	4
## HINDLIMBS										
I HINDLIMB(S) DAMAGED DURING PROCESSING	1	0	1	0	1	1	0	1	0	1
## METATARSALS										
I METATARSAL(S) MISSING (LOWER LIMB) - MECHANICAL DAMAGE	0	0	2	1	0	0	0	2	1	0
## HIND.PROXIMAL										
I PROXIMAL PHALANGES MISSING (HINDPAWS) - MECHANICAL DAMAGE	0	0	2	2	1	0	0	2	2	1
A PROXIMAL PHALANGES MISSING (HINDPAWS)	0	0	0	3	0	0	0	0	1	0
## HIND.MIDDLE										
I MIDDLE PHALANGES MISSING (HINDPAWS) - MECHANICAL DAMAGE	2	0	7	4	4	2	0	7	3	3
R TWO OR MORE MIDDLE PHALANGES (LOWER LIMB) NOT OSSIFIED	0	0	0	4	0	0	0	0	1	0
R ONE MIDDLE PHALANX (LOWER LIMB) NOT OSSIFIED	0	0	0	5	0	0	0	0	2	0
A MIDDLE PHALANGES MISSING (HINDPAWS)	0	0	0	5	0	0	0	0	3	0
## HIND.DISTAL										
I DISTAL PHALANGES MISSING (HINDPAWS) - MECHANICAL DAMAGE	3	0	8	5	4	3	0	7	3	3
A DISTAL PHALANGES MISSING (HINDPAWS)	0	0	0	0	0	0	0	0	0	0

SIGNIFICANTLY DIFFERENT FROM CONTROLS * P < 0.05, ** P < 0.01
ANOMALY CODE : A = Abnormality (malformation) I = Incidental R = Retardation V = Variation
CODE FOR DOSE GROUPS :

1	2	3	4	5
G(VEH) + G(VEH) VEHICLE CONTROL	G(VEH) + 100 S. REFERENCE COMP'D	25 T. + 100 S. LOW DOSE	100 T. + 100 S. HIGH DOSE	100 T. + G(VEH) CONTROL

Visceral Examination: Incidences of anomalies were similar among groups.

Malformations: Two to 5 fetuses per treatment group had a malformation. The incidence rate was slightly, but not significantly elevated in the HT/S group (Sponsor Table 10).

TABLE 10. SUMMARY INCIDENCE OF LITTERS AND FETUSES WITH FRANK MALFORMATIONS

A SEGMENT II TERATOLOGY STUDY OF No 40-7582/001 IN COMBINATION WITH No 11-7018 ADMINISTERED ORALLY TO PREGNANT NZW RABBITS

EXPERIMENT NUMBER : 00792

DOSE GROUPS : DOSAGES :	VEHICLE CONTROL 0(VEH) + 0(VEH)	REFERENCE COMP'D 0(VEH) + 100 S.	LOW DOSE 25 T. + 100 S.	HIGH DOSE 100 T. + 100 S.	CONTROL 100 T. + 0(VEH)
# EVALUABLE LITTERS	19	18	20	19	19
# EVALUABLE FETUSES	147	138	162	137	143
LITTERS AND FETUSES WITH ANY MALFORMATION(S)					
# LITTERS EVALUATED	19	18	20	19	19
# LITTERS AFFECTED N(%)	3 (15.8)	4 (22.2)	3 (15.0)	5 (26.3)	2 (10.5)
# FETUSES EVALUATED	147	138	162	137	143
# FETUSES AFFECTED N(%)	3 (2.0)	6 (4.3)	3 (1.9)	11 (8.0)	2 (1.4)
LITTERS AND FETUSES WITH GROSS MALFORMATION(S)					
# LITTERS EVALUATED	19	18	20	19	19
# LITTERS AFFECTED N(%)	0	1 (5.6)	0	3 (15.8)	1 (5.3)
# FETUSES EVALUATED	147	138	162	137	143
# FETUSES AFFECTED N(%)	0	1 (0.7)	0	5 (3.6)	1 (0.7)
LITTERS AND FETUSES WITH VISCERAL MALFORMATION(S)					
# LITTERS EVALUATED	19	18	20	19	19
# LITTERS AFFECTED N(%)	2 (10.5)	1 (5.6)	2 (10.0)	2 (10.5)	1 (5.3)
# FETUSES EVALUATED	148	130	161	134	141
# FETUSES AFFECTED N(%)	2 (1.4)	3 (2.3)	2 (1.3)	2 (1.5)	1 (0.7)
LITTERS AND FETUSES WITH SKELETAL MALFORMATION(S)					
# LITTERS EVALUATED	19	18	20	19	19
# LITTERS AFFECTED N(%)	1 (5.3)	2 (11.1)	2 (10.0)	3 (15.8)	0
# FETUSES EVALUATED	148	130	151	134	141
# FETUSES AFFECTED N(%)	1 (0.7)	2 (1.5)	2 (1.3)	5 (3.0)	0

ONLY FINDINGS CLASSIFIED AS ABNORMALITIES (frank malformations and malpositioning) ARE INCLUDED IN THIS TABLE
SIGNIFICANTLY DIFFERENT FROM CONTROLS: * P < 0.05 ** P < 0.01 *** P < 0.001

Three does had multiple malformed fetuses:

1 Sinemet only: 3 live fetuses, 4 resorptions. All live fetuses had hemorrhagic lungs.

1 HT/S doe: Five of nine fetuses were malformed. Four were missing middle or distal phalanges in the hindpaws, and one had persistent truncus arteriosus.

1 HT/S doe: Six live fetuses and one early resorption. Three of the six fetuses had cutaneous syndactyly.

Food consumption was significantly reduced in these does during the treatment period. The body weights of the fetuses were markedly lower than their littermates.

Toxicokinetics:

Plasma samples were obtained from satellite groups of animals (n = 2 per dose) on days 6 and 11 of gestation at 1, 3, 8 and 24 hr post-treatment. Samples were analyzed for tolcapone, L-DOPA and metabolites. Interpretation of the data is limited by the small sample size.

The dose-related increase plasma tolcapone levels was less than dose-proportional, particularly in terms of Cmax (Sponsor Table A). Slightly higher tolcapone AUCs were determined in the HT/S animals as compared to the tolcapone-only animals. Levels of the 3-O-methyl metabolite were similarly low in all treatment groups.

Tolcapone did not dramatically influence the Cmax or AUC of L-DOPA (Table C). Levels of 3-O-methyl-DOPA were markedly reduced by tolcapone. Carbidopa levels were not influenced by tolcapone.

Dose corrected exposures for tolcapone are approximately four-fold lower in rabbits as compared to rats.

Table A : Pharmacokinetic parameters of Ro 40-7592 (tolcapone)

	Cmax (µg/ml)	
Dosage group	Day 6	Day 11
3	1.89-3.74	2.73*
4	3.05-7.01	3.91-4.17
5	4.36-5.16	5.40-8.74

* single value

	AUC_{0-24} (h.µg/ml)	
Dosage group	Day 6	Day 11
3	12	11
4	42	36
5	26	29

Table C : Pharmacokinetic parameters of Ro 05-4759 (L-Dopa)

	Cmax (µg/ml)	
Dosage group	Day 6	Day 11
2	3.91-5.20	7.95-16.1
3	7.22-8.27	5.05-28.1
4	4.92-6.05	5.45-6.52

	AUC_{0-24} (h.µg/ml)	
Dosage group	Day 6	Day 11
2	23	34
3	34	65
4	33	34

Group 2 : 80 mg/kg L-Dopa plus 20 mg/kg carbidopa
Group 3 : 80 mg/kg L-Dopa plus 20 mg/kg carbidopa and 25 mg /kg Ro 40-7592
Group 4 : 80 mg/kg L-Dopa plus 20 mg/kg carbidopa and 100 mg /kg Ro 40-7592

C.5. Genotoxicity

a. Mutagenicity of Tolcapone in the Ames test
b. Mutagenicity of Tolcapone in combination with Sinemet in the Ames test
c. Mutagenicity of Tolcapone in E. coli WP2 uvrA - Extension of Ames test
d. Gene Mutation Assay (V79/HGPRT) of Tolcapone
e. Unscheduled DNA Synthesis with Tolcapone in Primary Cultures of Rat Hepatocytes
f. Chromosome Analysis in Human Lymphocytes Exposed to Tolcapone *In Vitro*
g. Mutagenicity of Tolcapone in Combination with Sinemet in the ML/TK Assay
h. *In vivo* Mouse Micronucleus Test
i. *In vivo* Mouse Micronucleus Test with Tolcapone in Combination with Sinemet

Conducted by: Hoffmann-La Roche, Ltd.
CH 4002 Basel
Switzerland

All studies complied with GLP.

C.5.a. Mutagenicity of tolcapone in the Ames test

Research Report #: B-153,807
Sponsor Volume: 50

Summary:

Tolcapone 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 were appropriate based on evidence of cytotoxicity; the cytotoxic concentration varied among the different strains. Positive controls produced the expected results.

Methods:

Drug concentrations: 5, 10, 50, 100, 250 µg/plate (Batch # Lab J. 3413.043 W prepared in DMSO). Concentrations were escalated as needed (375-1000 µg/plate) in resistant strains (TA97, TA1535, TA1538) to achieve cytotoxicity (reduction of his^+ revertants).

Strains/Positive controls/Vehicles:

Strain	Sensitivity	Positive Control	Conc./Vehicle
TA 1535	base-pair substitution	sodium azide	1.0 µg/plate in DMSO
TA 1537	"	ICR 191	1.0 µg/plate in DMSO
TA 1538	"	2-acetylaminofluorene	4.0 µg/plate in DMSO
TA 100	"	sodium azide	1.0 µg/plate in DMSO
TA 97	frameshift mutation	ICR 191	1.0 µg/plate in DMSO
TA 98	frameshift mutation	2-acetylaminofluorene	4.0 µg/plate in DMSO
TA 102	oxidizing/crosslinking agents	mitomycin C	0.4 µg/plate in water

Metabolizing System: S9 fraction prepared from phenobarbital/βnaphthoflavone-induced rats
2-aminoanthracene (4.0 µg/plate in DMSO) was also tested in all strains to verify S9 activity.

Test Conditions: Standard plate incorporation assay (2-day incubation)
Liquid preincubation assay
- 2-day incubation; four replicates for test compound and negative control, two replicates for positive control

Results:

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

In most cases, cytotoxicity occurred at concentrations of 250 µg/ml. In strains TA 1538, TA 1535 and TA 97 concentrations of 375-1000 µg/plate caused cytotoxicity, but no increase in mutation frequency.

TABLE 1 Salmonella mutagenicity test (Ames standard assay).
Mean values and standard deviations
For detailed data see appendix, table A1, A2, and A3

Experiment No	01	01	01	01	01	01	04	04
Activation	-S9	+S9	-S9	+S9	-S9	+S9	-S9	+S9
Strain	TA 1535	TA 1535	TA 1537	TA 1537	TA 1538	TA 1538	TA 1538	TA 1538
Concentration in µg /plate	Test substance: Ro 40-7592/000							
0.00	15 ± 6	10 ± 1	17 ± 9	13 ± 3	20 ± 2	27 ± 7	15 ± 5	23 ± 4
5.00	13 ± 3	9 ± 1	14 ± 2	12 ± 3	16 ± 4	21 ± 3	15 ± 2	21 ± 4
10.00	10 ± 2	9 ± 1	14 ± 2	11 ± 3	20 ± 5	29 ± 5	13 ± 3	24 ± 1
50.00	10 ± 2	11 ± 3	14 ± 12	10 ± 3	18 ± 2	24 ± 2	13 ± 5	20 ± 3
100.00	11 ± 5	11 ± 4	12 ± 3	11 ± 4	21 ± 3	27 ± 3	12 ± 2	24 ± 4
250.00	10 ± 2	7 ± 3	9 ± 2	8 ± 2	17 ± 7	21 ± 2	9 ± 2	16 ± 1

Experiment No	02	02	02	02	02	02	02	02
Activation	-S9	+S9	-S9	+S9	-S9	+S9	-S9	+S9
Strain	TA 97	TA 97	TA 98	TA 98	TA 100	TA 100	TA 102	TA 102
Concentration in mcg /plate	Test substance: Ro 40-7592/000							
0.00	256 ± 8	341 ± 24	22 ± 3	30 ± 4	89 ± 12	95 ± 13	222 ± 34	220 ± 14
5.00	261 ± 9	343 ± 24	22 ± 4	27 ± 10	85 ± 8	89 ± 9	204 ± 13	190 ± 11
10.00	260 ± 30	298 ± 25	23 ± 4	33 ± 2	87 ± 18	85 ± 20	204 ± 49	164 ± 7
50.00	243 ± 18	285 ± 20	27 ± 5	39 ± 6	85 ± 6	74 ± 13	152 ± 13	126 ± 5
100.00	259 ± 8	292 ± 10	22 ± 6	29 ± 3	78 ± 3	78 ± 10	91 ± 15	91 ± 10
250.00	265 ± 26	262 ± 12	1 ± 1	11 ± 3	46 ± 12	62 ± 8	20 ± 4	34 ± 5

TABLE 2 Salmonella mutagenicity test (Liquid preincubation assay).
Mean values and standard deviations.
For detailed data see appendix, table A4 and A5.

Experiment No	03	03	03	03	03	03
Activation	-S9	+S9	-S9	+S9	-S9	+S9
Strain	TA 1535	TA 1535	TA 1537	TA 1537	TA 1538	TA 1538
Concentration in µg /plate	Test substance: Ro 40-7592/000					
0.00	12 ± 3	13 ± 5	14 ± 5	13 ± 6	10 ± 3	18 ± 3
5.00	15 ± 2	17 ± 4	13 ± 2	15 ± 3	13 ± 3	24 ± 5
10.00	16 ± 5	14 ± 3	15 ± 3	16 ± 2	15 ± 2	22 ± 4
50.00	14 ± 3	15 ± 4	12 ± 2	16 ± 4	13 ± 4	23 ± 4
100.00	16 ± 5	14 ± 3	12 ± 4	13 ± 1	11 ± 1	20 ± 6
250.00	16 ± 4	19 ± 6	6 ± 2	9 ± 2	10 ± 4	15 ± 2

Experiment No	05	05	05	05	05	05	05	05
Activation	-S9	+S9	-S9	+S9	-S9	+S9	-S9	+S9
Strain	TA 97	TA 97	TA 98	TA 98	TA 100	TA 100	TA 102	TA 102
Concentration in µg /plate	Test substance: Ro 40-7592/000							
0.00	227 ± 32	232 ± 2	18 ± 4	30 ± 3	94 ± 5	93 ± 5	255 ± 16	271 ± 28
5.00	230 ± 11	227 ± 25	17 ± 3	28 ± 2	82 ± 13	93 ± 3	257 ± 9	269 ± 20
10.00	194 ± 20	232 ± 18	18 ± 2	24 ± 4	87 ± 8	84 ± 15	263 ± 12	280 ± 20
50.00	217 ± 14	214 ± 9	20 ± 3	27 ± 2	85 ± 16	89 ± 9	240 ± 10	245 ± 36
100.00	224 ± 4	219 ± 18	13 ± 4	29 ± 3	96 ± 18	84 ± 9	191 ± 13	276 ± 16
250.00	268 ± 12	219 ± 10	12 ± 6	18 ± 6	90 ± 10	78 ± 13	86 ± 10	190 ± 11

TABLE 3a Salmonella mutagenicity test (Ames standard assay).
Mean values and standard deviations.
For detailed data see appendix, table A6 and A7.

Experiment No	09	09	07	07	07	07
Activation	-S9	+S9	-S9	+S9	-S9	+S9
Strain	TA 97	TA 97	TA 1535	TA 1535	TA 1538	TA 1538
Concentration in µg /plate	Test substance: Ro 40-7592/000					
0.00	277 ± 14	325 ± 24	27 ± 1	14 ± 3	22 ± 1	32 ± 8
100.00	273 ± 20	302 ± 14	23 ± 3	13 ± 2	27 ± 4	10 ± 3
250.00	270 ± 11	279 ± 9	20 ± 1	12 ± 2	15 ± 3	20 ± 4
375.00	260 ± 6	284 ± 30	12 ± 3	11 ± 6	8 ± 4	13 ± 1
500.00	270 ± 14	271 ± 18	8 ± 1	8 ± 2	2 t ± 2	8 ± 4
750.00	258 ± 26	276 ± 13				
1000.00	182 ± 11	201 ± 23				

TABLE 3b Salmonella mutagenicity test (Liquid preincubation assay).
Mean values and standard deviations.
For detailed data see appendix, table A8 and A9

Experiment No	10	10	08	08	08	08
Activation	-S9	+S9	-S9	+S9	-S9	+S9
Strain	TA 97	TA 97	TA 1535	TA 1535	TA 1538	TA 1538
Concentration in µg /plate	Test substance: Ro 40-7592/000					
0.00	265 ± 16	289 ± 21	29 ± 6	16 ± 3	23 ± 2	22 ± 5
100.00	270 ± 16	270 ± 28	26 ± 4	15 ± 3	18 ± 3	18 ± 1
250.00	271 ± 23	259 ± 9	16 ± 7	10 ± 1	10 t ± 2	15 ± 2
375.00	298 ± 30	294 ± 30	13 ± 2	11 ± 2	6 t ± 2	11 ± 3
500.00	286 ± 16	259 ± 20	13 ± 3	T	T	5 ± 1
750.00	240 t ± 13	255 ± 13				
1000.00	73 t ± 17	182 ± 30				

T, t : Toxic effect (see corresponding Tables)

C.5.b. Mutagenicity of tolcapone in combination with Sinemet in the Ames test

Research Report #: B-163,287

Sponsor Volume: 51

Summary:

The combination of tolcapone and Sinemet 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 (1000 and 500 µg/plate in the standard and preincubation test, respectively) were appropriate based on evidence of cytotoxicity in most strains (TA 97 was resistant to toxicity); however, a rather large gap existed between the "no effect" and toxic concentrations. Positive controls produced the expected results.

Methods:

Drug concentrations: 5:4:1 ratio of tolcapone (Batch 40802440), L-DOPA, and carbidopa

standard assay: 31.6, 100, 316, 1000 µg/plate
Sinemet alone was tested at 500 µg/plate

preincubation assay: 15.8, 50, 158, 500 µg/plate
Sinemet alone was tested at 250 µg/plate

Note: The Summary Table 2 shown below indicates that the test concentrations of 250 and 500 µg/plate in the liquid preincubation assay contained Sinemet only, but the Appendix Tables of raw data and the text suggest that only the 250 µg/plate concentration contains Sinemet only.

Bacterial Tester Strains Positive Controls/vehicles:

Strain	Sensitivity	Positive Control	Conc./Vehicle
TA 1535	base-pair substitution	sodium azide	1.0 µg/plate in DMSO
TA 97	frameshift mutation	ICR 191	1.0 µg/plate in DMSO
TA 98	frameshift mutation	2-nitrofluorene	0.5 µg/plate in DMSO
TA 100	base-pair substitution	sodium azide	1.0 µg/plate in DMSO
TA 102	oxidizing/crosslinking agents	MMC	0.4 µg/plate in water

2-aminoamthracene (4.0 µg/plate in DMSO) was also tested in all strains to verify S9 activity.

Metabolizing System: The S9 fractions were prepared from phenobarbital/β-naphthaflavone-induced rats.

Results:

The combination of tolcapone and Sinemet did not increase mutation frequency above control values under any of the test conditions. Cytotoxic effects of the combination were evident in most strains as a reduction in the number of mutant colonies. The concentration at which cytotoxicity occurred varied among strains; TA 97 was resistant to toxicity at the highest test concentrations. Sinemet alone was not toxic at concentrations of 500 µg/plate in the standard assay and 250 µg/plate in the preincubation assay.

There was a relatively large gap between the two highest test doses of the combination in both assays. However, test concentrations between these levels would probably not have been useful, since tolcapone alone was shown to be cytotoxic in the previous study at concentrations between 250-500 µg/plate.

Spontaneous reversion frequencies on control plates were within historical control. The positive controls produced the expected increases in mutation frequency (data not shown).

Summary of the results of the reverse mutation assay using bacteria of the indicated strains.
Mean values and standard deviations.
For detailed data see the APPENDIX TABLE

1

Study No.: 92005 Experiment No.: 1 Experiment Starts: 30.05.95 Method: PRE
Name: Tolcapone/Sinemet

Strain Activation	TA1535 -S9	TA1535 +S9	TA97 -S9	TA97 +S9	TA98 -S9	TA98 +S9	TA100 -S9	TA100 +S9	TA102 -S9	TA102 +S9
Concentration µg/plate										
0.	13 ± 6	9 ± 1	193 ± 8	212 ± 11	26 ± 5	35 ± 1	82 ± 21	73 ± 3	376 ± 14	306 ± 18
31.6	22 ± 2	6 ± 4	197 ± 12	215 ± 11	33 ± 3	29 ± 4	69 ± 9	77 ± 2	303 ± 25	389 ± 37
100.	21 ± 2	9 ± 3	197 ± 7	230 ± 6	31 ± 7	30 ± 8	70 ± 16	66 ± 3	339 ± 10	429 ± 13
316.	11 ± 5	11 ± 6	213 ± 6	225 ± 7	29 ± 6	31 ± 6	71 ± 6	69 ± 17	310 ± 13	306 ± 19
500. *	16 ± 3.	9 ± 3	230 ± 9	241 ± 11	33 ± 9	40 ± 3	62 ± 8	77 ± 9	317 ± 22	441 ± 17
1000.	1 ± 1	6 ± 1	191 ± 3	176 ± 7	0 ± 0	6 ± 3	5 ± 3	7 ± 3	3 ± 3 t	91 ± 5

t : Toxic Effect (see corresponding tables)

*: Sinemet only

TABLE 2 Summary of the results of the reverse mutation assay using bacteria of the indicated strains. Mean values and standard deviations. For detailed data see the ATTACHED TABLE

Study No.: 82MS Experiment No.: 2 Name: Talampanel/Sinemet Experiment Start: 31.08.95 Method: PMI

Strain Activation	TA1535 -S9	TA1535 +S9	TA97 -S9	TA97 +S9	TA98 -S9	TA98 +S9	TA100 -S9	TA100 +S9	TA102 -S9	TA102 +S9
Concentration µg/plate										
0.	13 ± 2	14 ± 5	168 ± 12	230 ± 8	15 ± 2	20 ± 4	72 ± 16	77 ± 3	436 ± 29	376 ± 11
15.8	16 ± 6	12 ± 3	205 ± 16	253 ± 23	11 ± 6	19 ± 2	69 ± 11	71 ± 13	412 ± 33	367 ± 27
	13 ± 8	6 ± 2	233 ± 10	236 ± 6	13 ± 2	23 ± 1	83 ± 11	61 ± 15	402 ± 35	362 ± 14
158.	7 ± 2	10 ± 2	239 ± 25	271 ± 3	10 ± 2	21 ± 6	51 ± 6	76 ± 11	306 ± 3	304 ± 27
250. *	14 ± 1	12 ± 6	200 ± 10	255 ± 4	20 ± 5	20 ± 3	60 ± 8	72 ± 5	419 ± 26	290 ± 23
500. *	9 ± 1	7 ± 3	218 ± 25	276 ± 17	4 ± 2	16 ± 5	36 ± 5	56 ± 3	39 ± 8 t	271 ± 12

t, T : Toxic Effect (see corresponding tables)

*: Sinemet only

C.5.c. Mutagenicity of Tolcapone in E. coli WP2 uvrA - Extension of Ames test

Research Report #: B-159,633

Sponsor Volume: 51

Summary:

Tolcapone was not mutagenic at concentrations up to 250 μg/plate with or without S9 activation in this extension of the Ames test.

Methods:

Drug Concentrations: 5, 10, 50, 100, 250 μg/plate (Batch G PUL 606 090) in DMSO

Positive Controls: 4-Nitroquinolineoxide (1.0 μg/plate) in DMSO
2-Aminoanthracene (4.0 μg/plate) in DMSO

Tester Strain: E. coli WP2uvrA

Metabolizing System:

The S9 fractions were prepared from phenobarbital/β-naphthaflavone-induced rats.

Assay Conditions:

Both the standard Ames assay and the liquid preincubation modification were used.

Results:

Increases in mutant frequency and cytotoxicity were not evident under any of the test conditions. Higher doses were not tested since toxicity was encountered in the *S. typhimurium* strains at 250 μg/plate (in most cases).

Positive controls produced the expected results and background mutant frequencies were within the historical control range.

C.5.d. Gene Mutation Assay (V79/HGPRT) of Tolcapone

Research Report #: B-154,907

Sponsor Volume: 51

Summary:

Tolcapone did not induce forward mutations of the HGPRT locus in the Chinese hamster V79 cell line in the presence or absence of S9 activation.

The test dosage range used in this study was appropriate as cytotoxicity was achieved at relatively low concentration (25 µg/ml in the absence of S9; 400 µg/ml in the presence of S9). Positive controls produced the expected mutations.

Methods:

Drug Concentrations and Exposures:

Tolcapone (Batch G PUL 557 089) was dissolved in DMSO:

Experiments without metabolic activation
Protocol No.: 22M90/0, 22M90/3

Test Chemical	Substance Concentration µg/ml	
	22M90/0	/3
Solvent control: DMSO	0	0
Ro 40-7592/001	1	5*
Ro 40-7592/001	5	25**
Ro 40-7592/001	25*	100[1]**
Ro 40-7592/001	50[1]**	150[1]**
Ro 40-7592/001	100[1]**	200[1]**
Ro 40-7592/001	250[2]**	
Reference substance: EMS	80	80

* Colour of medium changed slightly immediately upon dosing
** Orange colour of medium immediately upon dosing
[1] Cell morphology changed at this dose level
[2] All cells became globular during treatment. Dose not taken for evaluation because criteria for minimal cell counts not fulfilled (Day 4)

Stock solution:
22M90/0: 51.5 mg Ro 40-7592/001 dissolved in 2.1 ml DMSO
22M90/3:108.1 mg Ro 40-7592/001 dissolved in 5.4 ml DMSO

Volume: 20 ml Ham F10-3
200 µl Solute resp. solvent

4.2 Experiments with metabolic activation
Protocol No.: 22M90/1, 22M90/2

Test Chemical	Substance Concentration µg/ml	
	22M90/1	/2
Solvent control: DMSO	0	0
Ro 40-7592/001	50[1]*	5
Ro 40-7592/001	100[1]*	50*
Ro 40-7592/001	200[1]*	200[1]*
Ro 40-7592/001	300[1]*	300[1]*
Ro 40-7592/001	400[1]*	400[2]*
Ro 40-7592/001		500[2]*
Reference substance: 2AAF	60	60

* Orange colour of medium immediately upon dosing
[1] Cell morphology changed at this dose level
[2] All cells became globular during treatment
[3] All cells became globular during treatment and did not longer-attach

Stock solution:
22M90/1: 231.1 mg Ro 40-7592/001 dissolved in 5.8 ml DMSO
22M90/2: 250.2 mg Ro 40-7592/001 dissolved in 5.0 ml DMSO

Volume: 8 ml Ham F10-3
2 ml CM S-9 mix (S-9: Batch B89/1 of September 8, 1989)
100 µl Solute resp. solvent

Test System:

The basis of this study is the presence/absence of hypoxanthine-guanine phosphoribosyl transferase in V79 cells. A mutation in the HGPRT locus results in cells which do not convert 6-thioguanine (6-TG) into a toxic metabolite, and thus survive treatment with media containing 6-TG. Mutants arise from base-pair substitutions, frameshifts, deletions, and chromosome rearrangements.

V79 Chinese hamster cells ($1x10^6$) were cultured for 24-30 hrs prior to exposure to test substance for 5 hr (with activation) or 16 hr (without activation). At 24 hr after cells were exposed to test substance, cells were reseeded at densities of 10^6/flask or higher if significant cytotoxicity occurred. Cells were subcultured every 2-3 days. On day 7, cells were exposed to medium containing 6-TG for mutant selection. An additional set dishes were used for determination of plating efficiency. Incubations were continued with media changes for 6-7 days. Cultures were fixed and stained and scored for mutant selection.

Cytotoxicity was assessed in separate experiments by measuring cell viability shortly after exposure to test compound.

Metabolizing System: S9 fraction prepared from Arochlor-induced rat (500 mg/kg given five days before tissue harvest)

Note: Difference from Ames assays using PB/β-Naphthaflavone induction

Statistics

Simultaneous group comparisons were by the Kruskal-Wallis test ($p \leq 0.05$). If a significant result was obtained, separate group comparisons versus control were by the Mann-Whitney U-test.

Results:

Cytotoxic effects (reduction in cell viability to ca. 20%) were evident with tolcapone concentrations as low as 25 µg/ml in cultures without metabolic activation (Table 1a, 1b). Tolcapone appeared to be less cytotoxic in the presence of S9 as cell viability was not markedly reduced at concentrations less than 400 µg/ml (Table 2a, 2b).

Mutant frequency was not significantly different from background in tolcapone-treated cultures in the absence of metabolic activation. In the presence of S9, mutation frequency was increased in cultures exposed to 50 µg/ml tolcapone, but not at any other concentration (Table 2a). This finding is considered spurious since it was not reproducible (Table 2b), and higher concentrations with comparable effects on cell viability did not increase mutation frequency.

EMS, the positive control for the experiments without activation, did not strongly increase mutation frequency in one of the two trials (Table 1b). The positive control for the experiments with S9 produced a moderate increase in mutation frequency.

Historical control data was not provided for comparison of the background mutant frequencies in the cells.

Table 1: : Total numbers of HGPRT-mutant cells, mutant frequency and viability of Chinese hamster V79 cells after a 16-hour exposure to RO 40-7592/001 without metabolic activation

Dose	Cell Viability Day 2			Day 7			HGPRT-Mutant Cells Day 7			
ug/ml.	No. (a)	Mean	RV %	No. (a)	Mean	CE %	No. (b)	Mean	Sign.	MF per 10^6 cells
0.	261 270 249 260	260.0	100	186 176 197 193	188.0	94	0 0 0 0 1 1 1 1 1 1 2 2	0.8		8.9
1.	278 267 287 300	283.0	100	231 240 261 233	241.3	121	0 0 0 1 1 1 1 1 1 2 2 2	1.0		8.3
5.	205 224 249 223	225.3	87	224 236 192 225	218.8	109	0 0 0 0 1 1 1 1 1 1 2 2	0.8		7.6
25.	65 57 54 57	58.3	22	226 221 198 241	221.5	111	0 0 0 0 0 0 0 0 1 1 1 1	0.3		3.0
50.	54 51 54 50	52.3	20	163 167 174 187	172.8	86	0 0 0 0 1 1 1 1 1 1 1 1	0.7		7.7
100.	83 92 100 89	91.0	35	209 208 204 219	210.0	105	0 0 0 0 0 0 1 1 1 1 1 2	0.6		5.6
Reference Substance : EMS										
0.	261 270 249 260	260.0	100	186 176 197 193	188.0	94	0 0 0 0 1 1 1 1 1 1 2 2	0.8		8.9
80.	242 211 244 224	230.3	89	176 151 164 151	160.5	80	9 11 13 13 15 16 17 18 20 20 21 22	16.3		202.5

Experiment Number : 22 M 90/0

a: 200 cells were plated per dish
b: 10^5 cells were plated per dish

X for P =< 0.05 XX for P =< 0.01

Trend: (+) increasing / (-) decreasing

Table 1b : Total numbers of HGPRT-mutant cells, mutant frequency and viability of Chinese hamster V79 cells after a 16-hour exposure to RO 40-7592/001 without metabolic activation

Dose	Cell Viability Day 2			Day 7			HGPRT-Mutant Cells Day 7			
ug/ml	No. (a)	Mean	RV %	No. (a)	Mean	CE %	No. (b)	Mean	Sign.	MF per 10^4 cells
0.	170 178 189 205	185.5	100	190 194 194 240	204.5	102	0 0 0 0 0 0 0 0 0 0 1 1	0.2		1.6
5.	172 162 174 192	175.0	94	162 156 150 153	155.3	78	0 0 0 0 0 0 0 0 0 0 0 1	0.1		1.1
25.	38 33 39 31	33.3	18	152 162 150 169	158.3	79	0 0 0 0 0 0 0 0 0 0 1 1	0.2		2.1
100.	39 27 32 31	32.5	17	139 131 141 130	135.3	68	0 0 0 0 0 0 0 0 0 1 1 1	0.5		3.7
150.	29 26 30 31	29.0	16	163 156 181 168	167.0	84	0 0 0 0 0 0 0 0 0 0 0 0	0.0		< 1.0
200.	38 29 24 22	28.3	15	130 113 146 144	133.3	67	0 0 0 0 0 0 0 0 0 0 0 1	0.1		1.3
Reference Substance : EMS										
0.	170 178 189 205	185.5	100	190 194 194 240	204.5	102	0 0 0 0 0 0 0 0 0 0 1 1	0.2		1.6
80.	138 146 149 142	143.8	77	134 152 142 135	140.8	70	1 1 1 1 1 1 3 3 4 5 5 6	2.7		37.9

Experiment Number : 22 M 90/3

a: 200 cells were plated per dish
b: 10^5 cells were plated per dish

X for P =< 0.05 XX for P =< 0.01

Trend: (+) increasing / (-) decreasing

Table 2: Total numbers of HGPRT-mutant cells, mutant frequency and viability of Chinese hamster V79 cells after a 5-hour exposure to RO 40-7592/001 and CM-S9-MIX

Dose	Cell Viability Day 2			Day 7			HGPRT-Mutant Cells Day 7			
ug/ml.	No. (a)	Mean	RV %	No. (a)	Mean	CE %	No. (b)	Mean	Sign.	MF per 10^6 cells
0.	221 215 215 252	225.8	100	210 214 218 223	216.3	108	0 0 1 1 1 1 1 2 2 2 2 2	1.3		11.6
50.	186 177 182 170	178.8	79	230 235 207 244	229.0	115	1 1 2 2 2 3 3 4 5 5 5 7	3.3	XX(+)	29.1
100.	173 164 180 177	173.5	77	189 186 208 204	196.8	98	0 0 0 1 1 1 1 2 2 2 3 3	1.3		13.6
200.	218 228 239 229	228.5	100	212 201 205 203	205.3	103	0 1 1 1 1 1 2 2 3 3 3 4	1.8		17.9
300.	191 171 202 160	181.0	80	171 170 166 173	170.0	85	0 1 1 1 1 2 2 2 2 3 3 6	2.0		23.5
400.	89 110 103 104	101.5	45	105 102 103 123	108.3	54	0 0 0 0 0 0 1 1 1 1 1 2	0.6		10.8
Reference Substance : 2AAF										
0.	221 215 215 252	225.8	100	210 214 218 223	216.3	108	0 0 1 1 1 1 1 2 2 2 2 2	1.3		11.6
60.	159 161 155 154	157.3	78	171 184 185 184	181.0	91	4 5 5 5 6 6 7 7 8 8 9 13	6.9		76.4

Experiment Number : 022 M 90/1

X for P =< 0.05 XX for P =< 0.01

a: 200 cells were plated per dish
b: 10^5 cells were plated per dish

Trend: (+) increasing / (-) decreasing