VII. REPRODUCTIVE AND DEVELOPMENTAL TOXICOLOGY:

Study title: Reproductive and Fertility Study with Memantine in the Rat

Key study findings:

- Memantine administration in Wistar/HAN rats at 2, 6, and 18 mg/kg/day by oral gavage (1X, 3X, and 9X the MRHD of 20 mg/d in a 60 kg patient on a BSA basis) resulted in reduced body weights and body weight gain in high dose F0 males and females, and decreased body weights in high dose F1 pups, F1 dams and F1 parent males
- Food consumption and testes weights reduced in the high dose F0 males
- NOAEL for maternal toxicity 6 mg/kg/day PO
- Memantine had no effect on male and female fertility in the F0 rats or their offspring
- NOAEL for adverse effects on fertility in rats 18 mg/kg/day

Study no.: 064214
Volume # 59, and page # 1
Conducting laboratory and location:

Date of study initiation: March 13, 1986
GLP compliance: yes (x) no ( )
QA reports: yes (x) no ( )
Drug Memantine, **lot #** R 7979, **radiolabel** Not applicable, **and % purity** 99.8%
Formulation/vehicle: Test article dissolved in distilled water

Methods:

Species/strain: Wistar/HAN Rats Ages 8-10 weeks, weights 200-250 g males and 180-200 g females)
Doses employed: 0, 2, 6, 18 mg/kg/day
Route of administration: Oral by gavage
Study design: Memantine was administered for 60 days prior to mating in the males and for 14 days prior to mating through mating, gestation (Part A females, 20 days post coitum) and lactation (Part B females, 20 days post partum) to necropsy in the females; F1 and F2 offspring were not treated; ½ F0 females (Part A) were sacrificed on gestation day 21 and ½ (Part B) sacrificed on Day 21 post partum
Number/sex/group: 24/sex/dose, 12 females/dose/timepoint
Parameters and endpoints evaluated:
Mortality: 2X daily
Clinical signs: 2X daily
Body weight: F0 Parents: Daily during treatment; F1 pups: Days 1, 4, 7, 14, 21 post partum; F1 dams: Days 0, 6, 11, 16, 21 post coitum and Days 1, 4, 7, 14, 21 post partum; F2 pups: Day 21 post partum
Food consumption: Weekly
F0 generation sacrificed on gestation day 21 (1/2 F0 females): F0 parent body weights at necropsy, cesarean parameters, fetal examination
Cesarean and fetal parameters: examination of ovaries and uteri, pregnancy status, number of corpora lutea, number and intrauterine position of implantations (live and dead

fetuses), early and late intrauterine deaths, fetal weights, external examination, sex, visceral and skeletal examination, internal abnormalities (Wilson technique)
Macroscopic examination and histology (ovaries, uterus, cervix, vagina, lesions, testes, epididymides, seminal vesicles, prostate, coagulating gland) performed on all adult animals
F0 and F1 Litter Parameters (1/2 F0 females allowed to litter normally): number of females paired, mated, pregnant, and reared pups, precoital time, percent animals mating, fertility index, conception rate, duration of gestation, gestation index, number of pups per dam at birth, implantations and postimplantation loss, breeding loss, F1 pup malformations, sex ratio, postnatal losses, development (pinna unfolding, incisor eruption, onset of coat development, eye opening, descent of testes, opening of vagina), pup behavioral tests (cliff avoidance, palmar grasp ability, negative geotaxis, exploratory locomotion pattern, righting reflex, photophobotaxis, direct pupillary reflex, hearing ability), F1 pup body weights, general findings during rearing period of F1 pups
F1 Generation Breeding F2 litters: Mortality, clinical signs, F1 terminal body weights, fertility, of F1 males and females, reproduction data of F1 dams (gestation duration, number of dams bearing and rearing young, number of pups/dam, implantations/dam, postimplantation loss, behavior of dams during parturition and lactation, body weights and food consumption of F1 dams
F2 pups: external examination, sex ratio, postnatal losses of F2 pups, body weights of F2 pups, general findings during rearing of F2 pups

Results:

Mortality: No treatment-related deaths in the F0 and F1 males and females
Clinical signs: No treatment-related effects in the F0 and F1 males and females
Body weight:
F0 males: marked, statistically significant decrease in body weight (BW) and body weight gain (BWG) at 18 mg/kg/day compared to controls from Days 10 (BW 4% lower than controls) through 60 (BW 11% lower than controls) before mating and Days 1 (BW 12% lower than controls) through 25 (BW 14% lower than controls) postpairing
F0 females: Statistically significant decrease in BW and BWG during pre-pairing Days 3 (BW 3% lower than controls) through 14 (BW 4% lower than controls), and gestation period (BWG 5.4% compared to 6.1% BWG in the controls) at 18 mg/kg/day compared to controls
F1 pups: Statistically significant decrease in mean BW on Days 1 (means of 5.7g compared to 6.0 g controls), 4 (7.9g compared to 8.4g controls), and 7 (11.4g compared to 11.9g controls) post partum in the high dose litters compared to controls, no effects on BWG
F1 Dams: Statistically significant decrease in BW at HD compared to controls on Days 0 (216g compared to 228g controls), 11 (251g compared to 264g controls), 16 (273g compared to 289g controls), and 21 (326g compared to 344g controls) post coitum and 1 (232g compared to 250g controls), 14 (277g compared to 291g controls), and 21 (269g compared to 283g controls) post partum, no treatment-related effects on BWG
F1 parent males: decreased BW at HD (mean 374.2 g) compared to controls (mean 403.4g) at necropsy
F2 pups: no treatment-related effects
Food consumption:
F0 males: decreased 11%-17% at 18 mg/kg/day during pre- and post-pairing periods compared to controls

F0 females: No statistically significant treatment-related effects
F1 dams: no treatment-related effects
Toxicokinetics: Not done

In-life observations: F0
No treatment-related effects on number of females mated and pregnant, mean precoital time, percent animals mating, fertility index, conception rate, gestation rate, gestation index, mean number of implantation sites per dam, mean number of corpora lutea per dam, percentage of embryonic resorptions, mean number of live fetuses per dam, preimplantation and postimplantation loss, mean number of dead fetuses, mean distribution of fetuses
No treatment-related effects in pup development in the F1 pups (pinna unfolding, incisor eruption, onset of coat development, eye opening, descent of testes, vagina opening)
No treatment-related effects on behavior (cliff avoidance, palmar grasp ability, negative geotaxis, exploratory locomotion pattern, righting reflex, photophobotaxis, direct pupillary reflex, hearing ability) in the F1 pups
No treatment-related effects on general findings during the rearing period in the F1 pups
F1 Fertility and Reproduction data: No treatment-related effects on number of F1 females mated, pregnant, bearing and rearing pups, percent animals mating, fertility index, conception rate, gestation index, mean duration of gestation, number of dams bearing and rearing young, mean number of pups per dam, mean number of implantations per dam, mean postimplantation loss, behavior of dams during parturition and lactation

Terminal and necroscopic evaluations:
F0 parent males: Statistically significant decrease in mean body weights at high dose compared to controls, decreased (12%) absolute (but not relative to body weight) testes weights at high dose
F0 parent females: No treatment-related macroscopic changes
F1 fetuses: no treatment-related malformations, effects on sex ratio, fetal body weights, fetal visceral abnormalities, skeletal malformations, in the Part A and Part B groups
Increased incidence of non-ossified cervical vertebrae in the high dose F1 fetuses (5%-33% fetuses compared to 0%-25% control fetuses for Cervical vertebrae 1-7, statistically significant increase for each of the 7 vertebrae), associated with decreased fetal body weight and retarded development
F1 pups: no treatment-related abnormalities
F1 parent males: decreased body weights at the high dose compared to controls, decreased absolute testes weights at the HD (7%, no effects on relative testes weights) compared to controls
F1 parent females: No abnormal, treatment-related findings
F2 fetuses: no treatment-related abnormal external findings, effects on sex ratios, postnatal losses, body weights, or general findings
F2 pups: No treatment-related findings

Study title: Embryotoxicity (Including Teratogenicity) Study with Memantine in the Rat

Key study findings:

- Memantine HCl was not teratogenic in rats at up to 9X the MRHD, under the conditions of this study

- Slight, but significant increase in the incidence of dumbell shaped thoracic vertebral body at the high dose of 18 mg/kg/day (4.2% compared to 2.3% in the controls, within the range of historical control data, not dose-related)
- Slight dose-related increase in the incidence of dumbell shaped cervical vertebral body, that was not statistically significant and was within the range of historical control data
- NOAEL for maternal toxicity 6 mg/kg/day (significantly reduced food consumption (25%), reduced body weight (5%), and reduced body weight gain (35%), and food consumption (7%-18%) at 18 mg/kg/day), although food consumption was slightly but significantly reduced up to 7% at this dose
- NOAEL for embryo-fetal development in rats was 18 mg/kg/day PO (9X the recommended human dose of 20 mg/day in a 60 kg patient on a BSA basis)

Study no.: 064225
Volume # 60, and page # 1
Conducting laboratory and location: [redacted]

Date of study initiation: December 2, 1986
GLP compliance: yes (x) no ( )
QA reports: yes (x) no ( )
Drug Memantine, **lot #** R7979, **radiolabel** Not applicable, **and % purity** 99.8%
Formulation/vehicle: Distilled water

Methods:

Species/strain: Wistar/HAN Rat [redacted], ages 11 weeks minimum, weights 184-235 g)
Doses employed: 0, 2, 6, and 18 mg/kg/day
Route of administration: Oral by gavage
Study design: Mated female rats were dosed daily on gestation days 6 through 15 (10 ml/kg); the females were sacrificed on gestation day 21 and the fetuses were removed; females and fetuses were examined macroscopically
Number/sex/group: 25 females/group
Parameters and endpoints evaluated: mortality (2x daily), clinical signs (2x daily), body weights (daily on gestation days 0 through 21), food consumption (gestation days 6, 11, 16, and 21), necropsy (macroscopic examination of all internal organs on gestation day 21), uterine/implantation data (uterine contents, pregnancy status, live fetuses, early and late intrauterine deaths, dead fetuses, position of fetuses in uterus, number of corpora lutea), fetal examination (sex, weights, gross external abnormalities, examination of viscera and brain, skeletal examination, for malformations and variations)

Results:

Mortality: No deaths
Clinical signs: No treatment-related effects
Body weight: BWG reduced 25% compared to controls at 6 mg/kg/day and 35% compared to controls at 18 mg/kg/day measured on gestation day 21, no statistically significant treatment-related differences in BW
Food consumption: Statistically significant, dose-related reduction at 6 (gestation days 6-11, 7% compared to controls) and 18 (gestation days 6-16, 7%-18% compared to controls) mg/kg/day

Toxicokinetics: Not done

In-life observations: Dams with live fetuses: 22, 25, 24, and 24 at 0, 2, 6, and 18 mg/kg/day (for calculations of body weight gain, food consumption and reproduction data).

Terminal and necroscopic evaluations:

Dams: No treatment-related effects in the macroscopic evaluation.

No treatment-related effects on reproduction data of dams, including uterine contents, pregnancy status, live fetuses, early and late intrauterine deaths, dead fetuses, position of fetuses in uterus, number of corpora lutea.

Offspring: No treatment-related effects were observed in the external and visceral examinations of the fetuses, sex ratios, and fetal body weights. There was a slight but statistically significant increase in incidence of dumbell shaped thoracic vertebral body at the high dose, that was considered to be incidental because the incidence was within historical control range and not dose-related. The incidence of malformations observed in the fetuses are presented in the following table:

Incidence of Malformations Observed in Fetuses of Female Rats Given Memantine*

Malformation	0 mg/kg/d	2 mg/kg/day	6 mg/kg/day	18 mg/kg/day
External Examination	**N=257**	**N=295**	**N=287**	**N=287**
Agnathia (inferior)		1 (0.3%)		
Visceral Examination	**N=127**	**N=142**	**N=143**	**N=144**
Hypoplastic left kidney		1 (0.7%)		
Dilated renal pelvis			1 (0.7%)	
Skeletal Examination	**N=130)**	**N=142**	**N=144**	**N=143**
Dumbell shaped cervical vertebra	38 (29%)	42 (30%)	52 (36%)	58 (41%)
Dumbell shaped thoracic vertebral body	3 (2.3%)	2 (1.4%)		6 (4.2%)
Agnathia inferior		1 (0.7%)		
Bipartate cervical vertebral body	7 (5%)	6 (4%)	8 (5.5%)	10 (7%)
Bipartite thoracic vertebral body			1 (0.7%)	
Bipartite sternebrae/abnormally ossified sternebrae	1		1 (0.7%)	1 (0.7%)

*Dosing proceeded by oral gavage daily during gestation days 6-15; percent incidence in parentheses)

Study title: Effect of Memantine on Pregnancy of the Rabbit

Key study findings:

- Doses studied (3, 10, and 30 mg/kg/day PO) were 3X, 10X, and 29X the MRHD of 20 mg/day in a 60 kg patient on a BSA basis
- Maternal toxicity included unsteady stance, bewildered appearance, lethargy, hunched posture, dilated pupils, pilo-erection, decreased fecal output, decreased mean body weights (6%-9% throughout gestation) and food consumption at the high dose of 30 mg/kg/day
- No treatment-related effects on the in-life observations, fetal anomalies, and fetal variations
- Non-dose-related malformations in the treated groups (including lumbar-sacral meningiocele, hydrocephaly, lumbar scoliosis, dilated aortic arch with interventricular septal defect,

bilateral lenticular opacity, and encephalocele at the low dose, sacral meningiocele with minimal protrusion of occipital region of the cranium at the mid-dose, and brachyury, lumbar scoliosis, sluggish with bilateral forelimb flexure and atelectatic lungs, retroesophageal right subclavian and carotid arteries, partial fusion of frontals, sutural bones and atelectatic lungs at the high dose) were within historical background incidence for the laboratory

- NOAEL for embryo-fetal toxicity in rabbits was 30 mg/kg/day PO, under the conditions of this study

Study no.: PTX 44/86149
Volume # 61, and page # 1
Conducting laboratory and location:

Date of study initiation: June 10, 1985
GLP compliance: yes (x) no ( )
QA reports: yes (x) no ( )
Drug memantine HCl, **lot #** R7245, **radiolabel** Not applicable, **and % purity** 99.7%
Formulation/vehicle: Test article dissolved in Water for Injection

Methods:

Species/strain: Non-pregnant New Zealand White rabbits (ages 13-16 weeks, weights 2.7-3.8 kg)

Doses employed: 0, 3, 10, and 30 mg/kg/day (0, 0.3, 1.0, and 3.0 mg/ml, 1 ml/kg). The doses were selected based on a pilot study in 6 non-pregnant rabbits at doses of 0, 3, 10, and 30 mg/kg/day. The results showed no treatment-related deaths. The clinical signs were unsteadiness and bewildered look, dilated pupils and pilo-erection, observed at the highest dose. There was a slight dose-related (1%-4%) decrease in body weight gain through Day 18, no differences from control on Day 19, and slight increase in body weight gain in the treated rabbits compared to controls from Days 20-29. No treatment-related effects on food consumption were observed. No treatment-related effects on the uterine/implantation parameters (pregnancy status, number of corpora lutea, number and intrauterine position of implantations, live fetuses, and embryonic/fetal deaths). One litter abortion at 3 mg/kg/day was not considered to be treatment-related.
In previous studies in rabbits, weight loss and bewildered appearance were observed at 35 mg/kg/day, and severe weight loss was observed at 50 mg/kg/day.

Route of administration: Oral by gavage

Study design: The rabbits were mated after 10 days acclimation, and then dosed daily from gestation days 6-18 (mating day considered pregnancy day 0). The rabbits were sacrificed by cervical dislocation on Day 29 for macroscopic examination and examination of the fetuses

Number/sex/group: 16 (LD, HD) - 17 (Controls, MD)

Parameters and endpoints evaluated: Morbidity and mortality (daily), clinical signs (daily), body weights (Days 6, 8, 10, 14, 19, 23, and 29), terminal studies (necropsy and uterine/implantation data including number of corpora lutea, number and intrauterine position of implantations, live fetuses, and embryonic/fetal deaths), and fetal data (weights, sex, internal visceral and external examination). Fetal structural deviations were defined as malformations (rare or lethal), anomalies (minor differences from normal), and variations (differences from normal with regular occurrence in the controls). Calculations were made for pre-implantation loss (#corpora lutea-#implantations X

100/#corpora lutea), post-implantation loss (#implantations-#live young X 100/#implantations).

Results:

Mortality: There were no treatment-related deaths. There were 4 deaths associated with intubation errors, pulmonary disorder, and perforated stomach.
Clinical signs: Unsteady stance, bewildered appearance, lethargy, hunched posture, dilated pupils, pilo-erection, cold ears, and decreased fecal output at the highest dose of 30 mg/kg/day.
Body weight: Mean body weights reduced 6%-9% throughout gestation at 30 mg/kg/day compared to controls.
Food consumption: Decreased on treatment days 6-13 at the highest dose compared to controls, no treatment-related differences in food consumption from Days 14-29.
Toxicokinetics: Not done
In-life observations: No treatment-related effects on pregnancy rate, number of live young, embryonic deaths, implants, corpora lutea, mean pre-implantation loss, and post-implantation loss.
Terminal and necroscopic evaluations:

Dams: No treatment-related effects on necropsy and uterine/implantation data (number of corpora lutea, number and intrauterine position of implantations, numbers of live fetuses, and embryonic/fetal deaths.
Offspring: Slight, non-significant decrease in mean litter size associated with slightly increased post-implantation loss at the high dose, due to loss of 9 deaths in one high dose litter. No treatment-related effects on sex ratios, mean fetal weights, mean litter weights. Malformations were observed in 10 fetuses in the treated groups. There were 0, 5, 1, and 4 malformations (0.0%, 4.6%, 0.6%, and 4.1%) at 0, 3, 10, and 30 mg/kg/day, within the historical background incidence (control groups in 7 studies (data provided) for the laboratory). The malformations included lumbar-sacral meningiocele, hydrocephaly, lumbar scoliosis, dilated aortic arch with interventricular septal defect, bilateral lenticular opacity, and encephalocele at the low dose, sacral meningiocele with minimal protrusion of occipital region of the cranium at the mid-dose, and brachyury, lumbar scoliosis, sluggish with bilateral forelimb flexure and atelectatic lungs, retroesophageal right subclavian and carotid arteries, partial fusion of frontals, sutural bones and atelectatic lungs at the high dose.

There were no treatment-related differences from controls in skeletal and gross visceral anomalies and skeletal variations.

Study title: Peri- and Postnatal Study with Memantine in the Rat

Key study findings:

- Memantine HCl, at doses of 2, 6, and 18 mg/kg/day (1X, 3X, and 9X the MRHD of 20 mg/d in a 60 kg patient on a BSA basis) by oral gavage in maternal rats, daily from gestation day 15 through lactation day 20
- Slight, non-statistically significant reduction in mean body weights and body weight gain, significantly reduced food consumption (9.5%) in HD dams compared to controls, during gestation period

- HD pup weights reduced approximately 5% throughout the post partum
- No treatment-related effects on pup development (pinna unfolding, incisor eruption, onset of coat development and eye opening)
- NOAEL for maternal toxicity 6 mg/kg/day
- NOAEL for prenatal and postnatal development in rats 18 mg/kg/day

Study no.: 064236
Volume # 61, and page # 86
Conducting laboratory and location: [redacted]

Date of study initiation: April 2, 1986
GLP compliance: yes (x) no ( )
QA reports: yes (x) no ( )
Drug Memantine HCl, **lot #** R 7979, **radiolabel** Not applicable, **and % purity** 99.8%
Formulation/vehicle: Test article dissolved in distilled water

Methods:

Species/strain: Female Wistar/HAN Rats [redacted], ages 12-13 weeks, weights 185-229 g, n=25/dose)
Doses employed: 0, 2, 6, and 18 mg/kg/day
Route of administration: Oral by gavage (10 ml/kg), once daily
Study design: The rats were mated, and the day spermatozoa were detected was designated as Day 0. The rats were dosed daily from gestation day 15 through lactation day 20 (post partum), and were sacrificed on lactation day 21
Number/sex/group: 25 mated females/group
Parameters and endpoints evaluated: Mortality (2X daily), clinical signs (2X daily), body weights (daily from days 0-21 post coitum, and days 1-21 post partum), food consumption (Days 6, 11, 15, and 21 post coitum, and Days 7 and 14 post partum), date of mating and parturition, number of pups, pup sex, viability and gross abnormalities, pup mortality, number of live pups on Days 1, 4, 7, 14, and 21 post partum, pup body weights (Days 1, 4, 7, 14, and 21 postpartum), pup developmental and behavioral abnormalities (pinna unfolding, incisor eruption, onset of coat development, eye opening), macroscopic examination of all female organs, necropsy of all dams and litters (post partum day 21) and those found dead and which lost litters.

Results:

Mortality: There was one death in a high dose (18 mg/kg/day) dam on gestation day 14 before dosing.
Clinical signs: No treatment-related clinical signs
Body weight: Slight, non-statistically significant reduction in mean body weight and body weight gain in the dams at 18 mg/kg/day compared to controls during treatment in the gestation period, no treatment-related differences during lactation period
Food consumption: Statistically significant reduction in mean food consumption in the dams at 18 mg/kg/day (9.5%) compared to controls during the gestation period treatment
Toxicokinetics: Not done

In-life observations:

Dams: No treatment-related effects on number of females paired, mated, pregnant, and bearing pups, number of implantations/dam, number of dams rearing pups, number of pups per dam, and breeding loss, duration of gestation, parturition and lactation behavior; absence of pregnancy was observed in 0, 1, 3, and 1 dam at 0, 2, 6, and 18 mg/kg/day, respectively; failure to give birth observed in 1 dam each at 0 and 2 mg/kg/day; failure to rear litters observed in 2, 1, 1, and 1 dam at 0, 2, 6, and 18 mg/kg/day, respectively.
Offspring: No treatment-related effects on external examination, and sex ratio during the 21-day post partum period, and general findings of pups during rearing.

Statistically significant ($p<0.05$) decrease in mean body weights of the pups throughout the 21-day post partum period at 18 mg/kg/day compared to controls, and occasionally significant reductions in body weights at 2 and 6 mg/kg/day compared to controls during the 21-day post partum period.

Mean Pup Weights (g, ± S.D.)*

Post-partum Day	0 mg/kg/day	2 mg/kg/day	6 mg/kg/day	18 mg/kg/day
1	5.8 ± 0.6	5.5 ± 0.6	5.6 ± 0.5	5.5 ± 0.6 (5.2%)
4	8.3 ± 1.1	8.0 ± 1.3	8.3 ± 0.9	7.9 ± 0.9 (4.8%)
7	12.1 ± 2.0	11.5 ± 1.6	12.0 ± 1.5	11.4 ± 1.6 (5.8%)
14	23.7 ± 4.3	22.5 ± 2.8	23.5 ± 3.0	22.4 ± 3.5 (5.5%)
21	37.2 ± 6.6	36.0 ± 4.5	37.2 ± 5.2	35.7 ± 5.8 (4.0%)

*Values in parentheses represent percent difference from controls

16 pups found dead on day 1 post-partum in 2 litters at 18 mg/kg/day: within historical range, and no statistically significant differences in mean number of pups/litter compared to control values by day 21 post-partum in this study.

No treatment-related effects on pup development (pinna unfolding, incisor eruption, onset of coat development and eye opening).

Terminal and necroscopic evaluations:
Dams: No treatment-related effects
Offspring: No treatment-related effects on necropsy findings after the 21-day post partum period; hernia of liver found in 1 control female pup, hydrocephalus found in one pup at 2 mg/kg/day.

VIII. SPECIAL TOXICOLOGY STUDIES:

Study title: MEMANTINE. Schirmer's test in the Beagle dog after oral administration. Study 442/008, Vol. 58, p. 55. Report Dated October 17, 1989.

Key study findings:
- No effects of oral memantine HCl on lacrimation in the Shirmer's test, at sequentially increasing doses from 4 to 20 mg/kg/day over 30 days
- Data not provided; conclusion cannot be supported by Agency review

Methods: Male and female (n=2/sex) Beagle dogs were administered oral memantine HCl at 4 mg/kg/day (Treatment Days 1-7), 8 mg/kg/day (Treatment Days 8-14), 12 mg/kg/day (Treatment Days 15-21), and 20 mg/kg (Treatment Days 22-30). One dog/sex was sacrificed at the end of the 12 mg/kg/day Treatment Period, and 1/sex was sacrificed at the end of the 20 mg/kg/day Treatment Period. The observations were clinical signs (daily), mortality (daily), body weight (weekly), food consumption (weekly), ophthalmological examination (pretest and end of each treatment period), and incidence of lacrimation (Schirmer's test, pretest and 4 hours after last dose of each treatment period).

Results: The sponsor reported a slight reduction in body weight during the 4 mg/kg/day treatment period and reduction in food consumption during the 20 mg/kg/day treatment period. There were no treatment-related ophthalmological abnormalities, and no treatment-related effects on lacrimation, although the individual data were not provided in the submission.

Study title: Investigation into the anterior eye segment lesions after chronic application of high dosages of memantine-HCl in two rat strains

Key study findings:

- Slit lamp examination showed no effects in albino rats, lens opacities in the anterior or posterior suture region and superficial cortex with waterclefts or faint vacuolations in 7/30 memantine-treated pigmented rats at end of treatment
- ——— photo-evaluation:
  - Increased corneal constant density and non-significant increase in lens capsular density in first half of treatment period, no change in optical density and nuclear density, no visible lens and corneal opacities in albino rats
  - Increased incidence of corneal density and lens capsular density compared to controls from mid-treatment to end of treatment, but no change in nuclear density in pigmented rats
- Results indicate significant cataract formation in pigmented but not in albino rats, at a dose associated with 30% mortality (180 mg/kg/day for 10 weeks, dietary) and considerable toxicity including reduced body weight gain and food consumption, and clinical signs of lethargy and aggressiveness
- The doses studied represented approximately 88X the MRHD of 20 mg in a 60 kg patient on a BSA basis
- NOAEL for ophthalmic toxicity was not determined in this study.

Study no: 93-0-40/94-0-44 AEOB
Volume # 58, and page # 58
Conducting laboratory and location: ———

Date of study initiation: September 9, 1993
GLP compliance: yes ( ) no (x)
QA report: yes ( ) no (x)
Drug Memantine HCl, **lot #** R 8825, **radiolabel** Not applicable, **and % purity** Not provided
Formulation/vehicle: Test article admixture in ——— Standard Diet No ———

Methods (unique aspects):
Dosing:

Species/strain: Experiment 1: Male Sprague Dawley rats
Experiment 2: Male Long Evans rats
#/sex/group or time point (main study): Experiment 1 n=11 controls and 31 treated; Experiment 2 n=10 controls and 30 treated
Satellite groups used for toxicokinetics or recovery: None
Age: Not provided
Weight: 196.5-217.0 g
Doses in administered units: 180 mg/kg/day
Route, form, volume, and infusion rate: Oral in the diet, continuous for 8 weeks in Experiment 1, and 10 weeks in Experiment 2

Observations and times:

Clinical signs: General health status weekly
Body weights: Weekly
Food consumption: Weekly
Ophthalmoscopy: Cornea and lens weekly, anterior eye segments with photography at baseline, 4 weeks and end of treatment
EKG: Not done
Hematology: Not done
Clinical chemistry: Not done
Urinalysis: Not done
Gross pathology: Not done
Organs weighed: Not done
Histopathology: Eyes only, end of treatment period
Toxicokinetics: Not done
Other: None

Results:

Mortality: 42% in the albino (Sprague Dawley) rats (1 in Wk 3, 1 in Wk 4, 2 in Wk 7, 5 in Wk 8, and 4 in Wk 9) and 30% in the pigmented (Long Evans) rats (1 in Wk 1, 3 in Wk 3, 1 in Wk 8, 3 in Wk 9, and 1 in Wk 10) over the entire treatment period
Clinical signs: Treatment-related lethargy alternating with aggressiveness in the albino and pigmented rats
Body weights: Treatment-related reduction in body weight gain in both rat species (33% in the albino and 38% in the pigmented rats at the end of the study)
Food consumption: Reduced in weeks 1, 3, 5, 6, 7 and 8 (up to 47% in the last week) in the albino rats, and in all treatment weeks (up to 34% in the last week) in the pigmented rats
Ophthalmoscopy:
Slit lamp examination: No effects in the albino rats, band-shaped corneal opacities in approximately 50% control and treated pigmented rats, lens opacities in 7/30 treated pigmented rats in the anterior or posterior suture region and superficial cortex, with waterclefts/faint vacuolations, persisting from mid-treatment to the end of the treatment period
photo-evaluation: Statistically significant treatment-related increase in constant density of the cornea in the first 4 weeks of treatment, and non-significant

increase in lens capsular density in the first 4 weeks of treatment, without changes in the optical density and nuclear density, and no visible opacities in the lens and corneas in the albino rats; in the pigmented rats there was an increase in incidence of corneal density and lens capsular density compared to controls from mid-treatment to the end of the treatment period, but no change in nuclear density in the treated rats
Electrocardiography: Not done
Hematology: Not done
Clinical chemistry: Not done
Urinalysis: Not done
Organ weights: Not done
Gross pathology: Not done
Histopathology: To be reported in a separate submission
Toxicokinetics: Not done

Study title:

Memantine Hydrochloride – 6 week oral (dietary administration) comparative study in the pigmented and albino rat. —— Study 442/011, Vol. 26, p. 1.

Memantine HCl – 6-week oral (dietary administration) comparative study in the pigmented and albino rat. Additional Histological Examination of the Brain. —— Study 442/011, Vol. 26, p. 262.

Memantine HCl – 6-week oral (dietary administration) comparative study in the pigmented and albino rat. Addendum 5 (Analytical results) and 6 (Histological procedure for the eyes). Study 442/011, Vol. 27, p. 1.

Key study findings:

- Greater lethality in the pigmented rats, 2 at MD (120 mg/kg/day) 7 at HD (180 mg/kg/day) than in the albino rats (1 at HD)
- Treatment-related clinical signs similar in both strains: hyperactivity, fur staining, piloerection
- Dose-related body weight reduction, similar in both strains
- Dose-related reduction in food consumption, similar in both strains
- Dose-related corneal lesions at MD and HD, bilateral corneal dystrophy in 1 HD albino, 3 MD and 3 high dose pigmented rats; slight increase in unilateral corneal dystrophy in pigmented rats, associated with vascularization and edema, slight increase in local opacities in anterior part of lens in HD pigmented rats
- Similar treatment-related organ weight changes in both strains; decreased thymus weights, spleen weights, Harderian gland weights
- Gross pathology: small spleen in 2 HD pigmented rats
- Histopathology: few species differences:
  - Vacuolation in the papilla and tubular cells in the cortex of kidneys in both strains at all doses
  - Slight species difference: increased vacuolation of tubular cells in papilla with foamy configuration in the pigmented rats and large vacuoles in the albino rats

  - Intraalveolar foamy cells (histiocytosis) with flocculent material in the lungs at all doses in both species
  - Hypoplasia with foamy cells in thymus at MD and HD
  - Foamy cells in liver, hypoplasia with foamy cells in the spleen, degeneration in testes, foamy cells in corneal epithelium of eye with erosion, keratitis/thinning of corneal epithelium and hypertrophy with foamy aspect of retinal pigmented epithelial cells in both strains at HD
- No necrosis or progressive degeneration in brain; foamy cytoplasmic vacuolation in the retrosplenial cortex in 2/5 high dose albinos (2/14 treated animals examined)
- Histological examination of eyes:
  - Hypertrophy with foamy aspect of pigment epithelium cells in the iris and retina in the high dose albino and mid-dose and high dose pigmented rats
  - Foamy aspect of the corneal epithelial cells, and erosion, keratitis, and thinning of the epithelium in the corneal stroma were in both strains with similar incidence and severity
  - Differences in memantine concentrations in the melanin-rich tissues in the pigmented rats were without corresponding increases in local pathology in the eye tissues.
- TK: increased plasma memantine concentrations with dosing duration at 120 and 180 mg/kg/day in both species, plasma drug levels higher in pigmented than in albino rats, higher memantine levels in skin, total eye, cornea, iris, vitreous body, retina and bulbus in pigmented compared to albino rats, no differences in memantine concentrations in tear fluid, Harderian gland, and lens between strains
- Higher memantine concentrations in skin and eye tissues than in plasma, increased memantine concentration in cornea, cornea is melanin-free and therefore increased concentration probably due to transfer of the drug from lens
- Greater than linear increase in tissue memantine concentrations with dose
- Test article intake confirmed at approximately intended doses
- Doses studied approximately 39X-88X MRHD of 20 mg/day, 60 kg patient, BSA basis
- NOAEL not determined

Study no: 442/011
Volume # 26 and 27, **and page #** 1
Conducting laboratory and location: [redacted]

Date of study initiation: July 31, 1991
GLP compliance: yes ( ) no (x)
QA report: yes (x) no ( )
Drug Memantine Hydrochloride, **lot #** R8825, **radiolabel** Not applicable, **and % purity** 100%
Formulation/vehicle: Test article admixed into basic powdered diet, homogeneity and stability of test article in the diet mixes performed

Methods (unique aspects):
Dosing:
Species/strain: Albino [redacted] rat, Pigmented Long Evans crl: (LE) rat
#/sex/group or time point (main study): 10 males/species/dose
Satellite groups used for toxicokinetics or recovery: None
Age: 6 weeks

Weight: Albino; 151.5-188.3 g, Pigmented: 118.6-185.6 g
Doses in administered units: 0, 80, 120, and 180 mg/kg/day
Route, form, volume, and infusion rate: Oral by admixture in the diet, continuously *ad libitum* for 44-45 days

Observations and times:

Clinical signs: 2X daily
Body weights: Weekly
Food consumption: Weekly
Ophthalmoscopy: Baseline, Week 4 (Control and HD), Week 6 (Control, MD, HD)
EKG: Not done
Hematology: Not done
Clinical chemistry: Not done
Urinalysis: Not done
Test article intake: Weekly (calculated as test article concentration X food intake/mean body weight
Gross pathology: External surface, all orifices, cranial cavity, carcass, external surface of brain, thoracic, abdominal and pelvic cavities and viscera, cervical tissues and organs
Organs weighed: Spleen, thymus
Histopathology: Adrenals, brain, eyelids, eyes, Harderian glands, kidneys, liver, lungs, thymus, thyroids
Toxicokinetics: Blood memantine levels analyzed on days 11, 25, and 45 (1 ml blood)
Other: Special histological examination of the brain and eye to investigate potential toxicity in the retrosplenial and cingulate cortices, and melanin-pigmented tissues

Results:

Mortality: 1 albino rat at 180 (day 42)
2 pigmented rats at 120 mg/kg/day (days 11, 25, during anesthesia)
7 pigmented rats at 180 mg/kg/day (days 11-43, 3 during anesthesia, 5 found dead)
Clinical signs: Hyperactivity in several albino rats at 80 mg/kg/day, most animals at 120 and 180 mg/kg/day in both strains, fur staining at 120 (albino) and 180 (both strains), piloerection at 180 (67% animals of both species, week 6 only)
Body weights: Dose-related reduction to similar extent in both strains (15%[albino]-17%[pigmented] at 80, 27%[albino]-29%[pigmented] at 120, 50%[albino]-55%[pigmented] at 180 mg/kg/day compared to controls, at end of study)
Food consumption: Dose-related reduction throughout study in albino rats (11%, 23%, 58% at 80, 120, and 180 mg/kg/day compared to controls at end of study) and pigmented rats (20%, 20%, and 50% at 80, 120, and 180 mg/kg/day compared to controls at end of study)
Ophthalmoscopy: Dose-related corneal lesions at 120 and 180 mg/kg/day: Bilateral corneal dystrophy in 3 pigmented MD rats, 1 albino and 3 pigmented HD rats; unilateral corneal dystrophy in 2 albino and 2 pigmented controls, 2 albino and 2 pigmented at MD, 1 albino and 5 pigmented at HD, with occasional vascularization and edema due to decreased lacrymal secretion or reduced blinking or high concentration of memantine metabolites in tear fluid and Harderian gland secretion; local opacities in anterior part of lens at HD (180 mg/kg/day, 5 albino and 2 pigmented rats) due to abnormal deposit (unilateral in 1 albino control, 3 pigmented controls)
Electrocardiography: Not done

Hematology: Not done
Clinical chemistry: Not done
Urinalysis: Not done
Organ weights: Decreased thymus weight at 80 (24% in pigmented rats), 120 (40% in albinos, 39% in pigmented) and 180 mg/kg/day (75% in albinos, 81% in pigmented), decreased spleen weight at 80 (25% in albinos, 21% in pigmented), 120 (39% in albinos, 33% in pigmented), and 180 mg/kg/day (55% in albinos, 71% in pigmented rats), decreased Harderian gland weight at 80 (22% in albinos), 120 (27% in albinos), and 180 (45% in albinos, 53% in pigmented) mg/kg/day
Gross pathology: Small thymus at 180 mg/kg/day (3 albino, 2 pigmented rats), small testes at 80 (1 albino), 120 (1 pigmented), and 180 (2 albino) mg/kg/day. Small spleen (2 pigmented rats) at 180 mg/kg/day
Histopathology: Observations similar in both strains:
80 mg/kg/day: changes in kidney (vacuolation in papilla in albino and pigmented rats) and lungs (minimal intraalveolar foamy cells / histiocytosis, with flocculent material)
120 mg/kg/day: Changes in kidney (vacuolation of tubular cells in cortex and papilla, in albino rats), lungs, thymus, eyes (retina), testes
180 mg/kg/day: Vacuolation of tubular cells in kidney (cortex and papilla, higher in albino than in pigmented rats), foamy cells in liver (both strains), hypoplasia with foamy cells in thymus (both strains) and spleen, intraalveolar foamy cells (histiocytosis) in lungs with flocculent material (both strains), degeneration in testes, foamy cells in corneal epithelium of eye with erosion (both strains), keratitis/thinning of corneal epithelium and hypertrophy with foamy aspect of retinal pigmented epithelial cells

Additional histological examination of the brain, —, was performed in 5 high dose albino, 9 high dose pigmented, and 5 each control albino and pigmented rats, to investigate potential toxicity by memantine in the cingulate and retrosplenial cortices (see — report No. 32191C, August 3, 1992). No necrosis or progressive degeneration was observed in any region, in the controls and high dose rats. The results showed occasional cells with perinuclear vacuolation in the retrosplenial cortex (1 control), foamy cytoplasmic vacuolation in the retrosplenial cortex (2/5 HD albinos [total: 2/14 treated animals]), occasional cells with condensed nuclei in the cingulum (control and treated), and intrafibrillary vacuolation (considered artifactual).

A special histological examination of the eyes was performed by — — see Report No. Add. 5 to 442/011, December 1992), based on observations in previous studies of affinity of memantine for melanin *in vitro* and in whole body autoradiography in pigmented rats, high drug content in pigmented tissues, and slow release of memantine from pigmented tissues. The toxicokinetic analysis included samples from eye tissues, tear fluid, Harderian gland, skin, and plasma from pigmented and albino rats. The tissues examined microscopically included the cornea, iris, lens, bulbus, vitreous body, and retina. The results showed hypertrophy with foamy aspect of pigment epithelium cells in the iris and retina in the high dose albino and mid-dose and high dose pigmented rats. Foamy aspect of the corneal epithelial cells, and erosion, keratitis, and thinning of the epithelium in the corneal stroma were observed in both strains with a similar incidence and severity. These changes are attributed to increased local concentrations of memantine in tear fluid, and not to increased melanin binding of memantine, because the histopathological abnormalities and tear fluid memantine concentrations were similar in both strains. The differences in memantine concentrations in the

melanin-rich tissues in the pigmented and albino rats were not associated with increased local pathology in the eye tissues.

Toxicokinetics: The mean plasma memantine concentrations are presented in the following table:

Mean Plasma Memantine Concentrations (mcg/ml) in Albino and Pigmented Rats Treated Orally (Dietary) for 6 Weeks (±S.D.)

Group	80 mg/kg/day	120 mg/kg/day	180 mg/kg/day
Albino			
Day 11	941 ± 268	1871 ± 832	2724 ± 651
Day 25	1844 ± 452	3046 ± 756	4462 ± 567
Day 45	1674 ± 1001	3180 ± 1098	5780 ± 1809
Pigmented			
Day 11	1521 ± 474	2249 ± 596	4049 ± 454
Day 25	1299 ± 385	2694 ± 642	4498 ± 668
Day 45	1463 ± 706	3321 ± 1382	8471 ± 2089

The results showed increased plasma memantine concentrations with dosing duration at 120 and 180 mg/kg/day in both albino and pigmented rats. The plasma drug levels were higher in the pigmented rats than in the albino rats on Day 11 (all doses) and Day 45 (180 mg/kg/day) only.

The mean tissue memantine concentrations are presented in the following table:

Mean Tissue Memantine Concentrations (mg/kg, except plasma concentration in mg/ml) on Day 45 in Albino and Pigmented Rats Treated Orally (Dietary) for 6 Weeks (±S.D.)

Tissue	Albino	Pigmented
	80 mg/kg/day	**80 mg/kg/day**
Plasma (mg/ml)	1.7 ± 1.0	1.5 ± 0.7
Tear fluid	24.9 ± 5.8	18.2 ± 6.1
Skin	37.4 ± 14.4	53.6 ± 16.8
Harderian gland	519.5 ± 81.0	380.7 ± 78.8
Total eye	13.1 ± 7.9	89.4 ± 12.7
Cornea	6.6 (pooled samples)	13.1 (pooled samples)
Iris	48.6 (pooled samples)	2304.4 (pooled samples)
Lens	3.1 (pooled samples)	3.5 (pooled samples)
Vitreous body	2.8 (pooled samples)	7.6 (pooled samples)
Retina	20.0 (pooled samples)	35.7 (pooled samples)
Bulbus	12.8 (pooled samples)	302.2 (pooled samples)
	120 mg/kg/day	**120 mg/kg/day**
Plasma (mg/ml)	3.2 ± 1.1	3.3 ± 1.4
Tear fluid	36.6 ± 7.9	40.8 ± 22.8
Skin	92.1 ± 43.9	185.0 ± 131.6
Harderian gland	680.5 ± 164.5	636.6 ± 146.7
Total eye	33.1 ± 20.3	210.1 ± 34.2
Cornea	29.8 (pooled samples)	86.3 (pooled samples)
Iris	172.8 (pooled samples)	6340.1 (pooled samples)
Lens	7.0 (pooled samples)	8.4 (pooled samples)
Vitreous body	6.7 (pooled samples)	18.4 (pooled samples)
Retina	119.0 (pooled samples)	198.3 (pooled samples)
Bulbus	71.0 (pooled samples)	607.3 (pooled samples)

	180 mg/kg/day	180 mg/kg/day
Plasma (mg/ml)	5.8 ± 1.8	8.5 ± 2.1
Tear fluid	168.0 ± 82.8	114.7 ± 25.2
Skin	573.8 ± 285.0	996.2 ± 530.2
Harderian gland	981.0 ± 325.0	859.4 ± 198.3
Total eye	127.6 ± 21.0	394.3 ± 26.0
Cornea	229.8 (pooled samples)	insufficient # survivors
Iris	2308.8 (pooled samples)	insufficient # survivors
Lens	24.1 (pooled samples)	insufficient # survivors
Vitreous body	21.7 (pooled samples)	insufficient # survivors
Retina	260.9 (pooled samples)	insufficient # survivors
Bulbus	241.0 (pooled samples)	insufficient # survivors

The results of the tissue memantine measurements showed higher memantine levels in pigmented than in albino skin, and higher concentrations in total eye, cornea, iris, vitreous body, retina and bulbus in the pigmented rats compared to the albino rats. There were no differences in memantine concentrations in tear fluid, Harderian gland, and lens between the two rat strains. The tissues studied had higher concentrations of memantine than did plasma, indicating accumulation, presumably to melanin pigments. The increased memantine concentration in cornea, which is melanin-free, is probably due to transfer of the drug from the lens. Overall, there was a greater than linear increase in tissue memantine concentrations with dose.

Test article intake: Albino rat:
72.15-86.64 mg/kg/day in 80 mg/kg/day group
100.26-129.17 mg/kg/day in 120 mg/kg/day group
126.33-197.17 mg/kg/day in 180 mg/kg/day group
Pigmented rat:
76.62-96.55 mg/kg/day in 80 mg/kg/day group
121.40-130.31 mg/kg/day in 120 mg/kg/day group
156.40-190.17 mg/kg/day in 180 mg/kg/day group

Study title: A comparative optical toxicity study of —— Y7017 by dietary administration in albino (SD) and pigmented (Long Evans) rats

Key study findings:

- Deaths in 4 HD albinos (sacrificed moribund) and 3 HD pigmented rats (found dead)
- Treatment-related clinical signs predominantly CNS origin: aggressiveness, hyperactivity, tremor, prolapsed penis, emaciation, piloerection, and decreased activity in both species; Hyperactivity, aggressiveness, prolapsed penis, and piloerection in both species at HD during 1st week of recovery
- Body weight gain reduced at MD from Week 2 to end of dosing in albinos and throughout dosing in pigmented; At HD decreased body weights and body weight gain in both species, reversed in albinos, and in 2/6 of pigmented rats
- Food consumption reduced at the MD and HD during treatment and reversed during recovery in both species
- Test article intake slightly below intended doses

- No species differences in ocular toxicity: eyeball opacities in 1 rat each in HD albinos on Day 6 and Week 4, HD pigmented in Week 4, and MD albinos in Week 6; At HD in Week 6, 5 albinos and 3 pigmented rats with opacities; no lesions observed after recovery
- Hematology; in both species increased hematocrit and hemoglobin, and decreased platelets, leukocytes and lymphocytes at MD and HD; Erythrocytes and segmented neutrophils increased and eosinophils decreased at the HD in albinos and MD and HD pigmented rats; Ratios of segmented neutrophils and monocytes, and ratio of monocytes increased at the MD and HD in the albinos, and segmented neutrophils and monocytes were increased and eosinophils decreased at HD in the pigmented rats
- Clinical chemistry: increased AST, ALT, CPK in MD and HD albinos and HD pigmented; At HD only LDH increased in both species, A/G ratio increased and total protein, albumin and Ca decreased in albinos, and total bilirubin and IP increased and glucose decreased in pigmented rats
- Organ weights similar in both species at MD and HD; decreased absolute and relative spleen weights, increased relative kidney, adrenal, and lung weights; also, absolute and relative thymus weights decreased and relative liver weights increased in MD and HD albinos, and relative heart weights increased in MD and HD pigmented rats; HD effects in both species were decreased absolute testes weights and increased absolute lung weights; relative heart weights increased in the HD albinos and absolute and relative brain weights increased, absolute adrenal weights increased, and absolute and relative thymus weights decreased in pigmented rats; after recovery, absolute and relative testes weights decreased in HD albinos, absolute and relative spleen weights decreased and lung weights increased in HD pigmented rats
- Gross pathology: No treatment-related effects at LD; MD effects were in albinos only, including small thymus, spleen and mesenteric lymph nodes; HD dose effects in both species; lung discoloration, small thymus, spleen, testes, prostate, seminal vesicles, mesenteric lymph nodes, epididymis, decreased adipose tissue, and dry subcutaneous tissue; in HD albinos only were emaciation, and large kidneys and adrenals; after recovery small testes and epididymides in the albinos
- Histopathology: no treatment-related effects in the eye, optic nerve and Harderian gland in either species; Both HD albinos and pigmented rats showed swollen Kupffer cells in the liver, vacuolar degeneration and dilatation of the renal tubules, foamy macrophages and eosinophilic material in the lung alveoli, degeneration and necrosis of muscle fibers, mononuclear cell infiltration in the skeletal muscles, vacuolation of nerve cells in the cerebrum, pons, and cerebellum (Purkinje cells), involution and foamy macrophages in the thymus, follicular atrophy and foamy macrophages in the spleen, and hypertrophy of the adrenal zona fasciculata; HD albinos showed atrophy of the tubular epithelium and necrosis of the collecting tubules in the kidneys; HD pigmented rats showed atrophy of hepatocytes; After recovery no abnormalities in pigmented rats, tubular regeneration in kidneys and decreasing severity of foamy macrophages in lungs in albinos
- Dose related increase in SUN Y7017 plasma concentrations in albino (2.4 and 3.84 mcg/ml at 120 and 160 mg/kg/day, respectively) and pigmented (1.82 and 3.93 mcg/ml at 120 and 160 mg/kg/day, respectively) rats, without species differences
- Conclusions: no toxicologically relevant differences between the albino and pigmented strains, and no treatment-related ocular toxicity in either species in this study
- Doses studied were approximately 58X and 78X MRHD of 20 mg in a 60 kg patient on a BSA basis

- NOAEL not determined in this study (<120 mg/kg/day)

Study no: ZR0001
Volume # 27, and page # 53
Conducting laboratory and location:

Date of study initiation: January 26, 2000 (main study)
GLP compliance: yes (x [Japan]) no ( )
QA report: yes (x) no ( )
Drug SUN Y7017, **lot #** 1798102, **radiolabel** not applicable, **and % purity** 99.2%
Formulation/vehicle: Test article admixture in powder diet ——— homogeneity and concentration confirmed

Methods (unique aspects):
Dosing:

Species/strain: Male Long Evans (pigmented) rats ——
Male Crj:CD(SD)IGS albino rats ———
#/sex/group or time point (main study): 8, 10, and 13 pigmented males at 0, 120, and 160 mg/kg/day, and 10, 10, and 14 albino males at 0, 120 and 160 mg/kg/day
Satellite groups used for toxicokinetics or recovery: 5 control and 6 HD males of each species necropsied at end of 4-week recovery period
Age: 6 weeks
Weight: SD (albinos): 196.2-223.5 g; LE (pigmented): 142.1-1889.8 g
Doses in administered units: 0, 120, and 160 mg/kg/day
Route, form, volume, and infusion rate: Oral by admixture in the diet, continuous ad libitum for 6 weeks

Observations and times:

Clinical signs: Daily
Body weights: Baseline and 3X/week, 2X/week during recovery period
Food consumption: Baseline and 3-day periods 2X/week
Test article intake: Calculated per week (memantine — X mean food consumption / mean body weight)
Ophthalmoscopy: Baseline, Treatment Weeks 4 and 6, Recovery Week 4
EKG: Not done
Hematology: End of 6-week treatment
Clinical chemistry: End of 6-week treatment
Urinalysis: not done
Gross pathology: External and internal organs
Organs weighed: Brain, thymus, heart, lungs, bronchus, liver, spleen, kidneys, adrenals, and testes
Histopathology: Brain, thymus, heart, lungs, bronchus, liver, kidneys, spleen, adrenals, testes, eye, optic nerve, Harderian gland, skeletal muscle, pancreas, stomach, duodenum, macroscopically abnormal organs or related organs and tissues, urinary bladder, femoral bone marrow, and sciatic nerve
Toxicokinetics: End of 6-week treatment
Other:

Results:

Mortality: SD (albino rats): 4 HD rats sacrificed moribund (Days 16, 23, 34, 38),
LE (pigmented rats): 3 HD rats found dead (Days 11, 37, 42)

Clinical signs:

Albino rats sacrificed moribund at HD, the signs were smudged perinasal and perioral areas, piloerection, emaciation, decreased activity, aggressiveness, opacity of eyeball, hyperactivity, bradypnea, tremor, prolapsed penis, staggering gait

Pigmented rats found dead at HD: hyperactivity, aggressiveness, tremors, prolapsed penis, bradypnea, tremors

Surviving albino rats: During treatment: aggressiveness, hyperactivity, piloerection, prolapsed penis, emaciation, decreased activity at the HD, and sporadic hyperactivity, aggressiveness at MD; During withdrawal: hyperactivity, aggressiveness, prolapsed penis, piloerection during first withdrawal week

Surviving pigmented rats: hyperactivity, aggressiveness, tremor at the MD and HD, and additionally, prolapsed penis, emaciation, bite wounds, swelling of foot pad, paralytic gait, decreased activity, necrosis of tip of tail at HD; during withdrawal period hyperactivity, aggressiveness, prolapsed penis during first 5 days

Body weights: SD (albino rats): Reduced body weight gain from the second week through end of treatment at MD (-24% compared to controls at end of treatment), decreased body weight (-47% compared to controls, end of treatment) and body weight gain at HD throughout treatment, reverse during recovery period

LE (pigmented rats): Reduced body weight gain throughout treatment at MD (-16% compared to controls, end of treatment), decreased body weights (-36% compared to controls, end of treatment) and body weight gain throughout treatment at HD, reversed in 2/6 rats during the recovery period

Food consumption: SD (albino rats): reduced at MD and HD during treatment, reversed during recovery period

LE (pigmented rats): Reduced at MD and HD during treatment, no difference during recovery period

Test article intake: Slightly below intended doses, at means of 111.3 and 141.8 mg/kg/day in the albino rats in 120 and 160 mg/kg/day groups, and 112.7 and 152.5 mg/kg/day in the pigmented rats in 120 and 160 mg/kg/day groups

Ophthalmoscopy: SD (albino rats): eyeball opacity in 1/19 HD animals in Day 6, 1/18 HD animals in Week 4, 1/10 MD and 5/17 HD animals in Week 6, no lesions after recovery period

LD (pigmented rats): Eyeball opacity in 1/19 HD rats in Week 4, 3/19 HD rats in week 6, no lesions after recovery period

Electrocardiography: Not done

Hematology: SD (albino rats): Increased hematocrit (4-5%), hemoglobin (4-5%), ratio of segmented neutrophils (64-200%), and ratio of monocytes (40-100%), and decreased platelets (20-35%), leukocytes (34-43%) and lymphocytes (41-58%) at MD and HD, increased erythrocytes (9%) and segmented neutrophils (36%), and decreased eosinophils (100%) at HD

LE (pigmented rats): increased erythrocyte count (4-5%), hematocrit (4-6%), hemoglobin (3-5%), decreased platelets (14-28%), leukocytes (22%), lymphocytes (25-48%) at MD and HD, and increased segmented neutrophils (130%) and monocytes (50%) and decreased eosinophils (50%) at HD

Clinical chemistry: SD (albino rats): increased AST (120-813%), ALT (13-189%), CPK (59-207%) at MD and HD, increased LDH (109%), A/G ratio (15%) and decreased TP (11%), ALB (3%) and Ca (4%) at HD
LE (pigmented rats): increased AST (578%), ALT (60%), LDH (100%), CPK (179%), Total Bilirubin (12%), and IP (23%) and decreased glucose (16%) at the HD
Urinalysis: Not done
Organ weights: SD (albino rats): decreased absolute and relative thymus and spleen weights, increased relative liver, kidney adrenal and lung weights at MD and HD, increased absolute lung and decreased absolute testes weights and increased relative heart weight at HD; after recovery period decreased absolute and relative testes weights at the HD
LE (pigmented rats): decreased absolute and relative spleen and increased relative heart, kidney, adrenal and lung weights at MD and HD; increased absolute and relative brain and decreased absolute and relative thymus weights and decreased absolute testes and increased absolute adrenal and lung weights at the HD; after recovery period decreased absolute and relative spleen and increased absolute and relative lung weights at the HD
Gross pathology: SD (albino rats): increased incidence of small thymus spleen and mesenteric lymph nodes at the MD, lung discoloration, small thymus, spleen, testes, prostate, seminal vesicles, decreased adipose tissue, dry subcutaneous tissue, emaciation, small mesenteric lymph nodes and epididymis, large kidneys and adrenals at the HD; no treatment-related effects observed after the recovery period, except small testes and epididymis
LE (pigmented rats): No treatment-related effects at the LD and MD; at the HD increased incidence of lung discoloration, small thymus, spleen, mesenteric lymph nodes, prostate, seminal vesicles, decreased adipose tissue, dry subcutaneous tissue, small epididymis and testes; no treatment-related effects after the recovery period
Histopathology: SD (albino rats): No treatment-related effects in eye, optic nerve, Harderian gland; swollen Kupffer cells in liver (7/14 rats vs 0/10 in controls), vacuolar degeneration (10/14 vs 0/10 controls) and dilatation of renal tubules (5/14 vs 0/10 controls), atrophy of tubular epithelium (6/14 vs 0/10 controls) and necrosis of collecting tubules (3/14 vs 0/10 controls) in kidneys, foamy macrophages (14/14 vs 3/10 controls) and eosinophilic material (12/14 vs 0/10 controls) in lung alveoli, degeneration (13/14 vs 0/10 controls) and necrosis of muscle fibers, mononuclear cell infiltration in skeletal muscles (12/14 vs 1/10 controls), vacuolation of nerve cells in cerebrum (12/14 vs 0/10 controls), pons (7/14 vs 0/10 controls), and cerebellum Purkinje cells (14/14 vs 0/10 controls), involution and foamy macrophages in thymus (13/14 vs 0/10 controls), follicular atrophy (14/14 vs 0/10 in controls) and foamy macrophages (7/14 vs 0/10 controls) in spleen, hypertrophy of adrenal zona fasciculata (4/14 vs 0/10 controls) at HD; after recovery period tubular regeneration in kidneys, foamy macrophages in lungs of lower severity than during treatment period
LE (pigmented rats): No treatment-related effects in eye, optic nerve, Harderian gland; swelling of Kupffer cells (2/13 vs 0/8 controls) and atrophy (1/13 vs 0/8 controls) of hepatocytes, vacuolar degeneration (7/12 vs 0/8 controls) and dilatation (1/12 vs 0/8 controls) of kidney tubules, foamy macrophages (13/13 vs 0/8 controls) and eosinophilic material (11/13 vs 0/8 controls) in lung alveoli, degeneration (7/13 vs 0/8 controls) and necrosis of muscle fibers, mononuclear cell infiltration (5/13 vs 0/8 controls) in skeletal muscles, vacuolation of nerve cells in cerebrum (13/13 vs 0/8 controls), pons (13/13 vs 0/8 controls) and cerebellum Purkinje cells (13/13 vs 0/8 controls), involution (7/12 vs

0/8 controls) and foamy macrophages (3/12 vs 0/8 controls) in thymus, follicular atrophy (8/12 vs 0/8 controls) and foamy macrophages (2/12 vs 0/8 controls) in spleen, hypertrophy of adrenal zona fasciculata (3/12 vs 0/8 controls) at HD; No abnormalities after recovery period

Toxicokinetics: Dose related increase in plasma SUN Y7017: 2.40 and 3.84 mcg/ml at 120 and 160 mg/kg/day in the SD rats and 1.82 and 3.93 mcg/ml in the LE rats; no differences between species in plasma SUN Y7017 concentrations

Study title: 13-Week Dose-Range Finding Study of Memantine HCl in B6C3F1 Mice by Administration in the Diet (To Determine the Maximum Tolerated Dose-Level for a Long-Term Feeding Study in Mice) – Supplementary Histopathology Report

Key study findings:

- Minimal to moderate cytoplasmic vacuolization in the brain stem and cerebellum in 5/5 male mice and minimal neuronal necrosis in the cerebellum in 2/5 male mice at 320 mg/kg/day (daily for 13 weeks)
- No differences in incidence of minimal vacuolization in the cingulate and retrosplenial cortices in the control and treated mice
- LOEL for neuronal vacuolation and necrosis by memantine in mouse brain stem and cerebellum 320 mg/kg/day (78X the MRHD of 20 mg/d in a 60 kg patient on a BSA basis)

Study no: 7196/92
Volume # 25, and page # 1
Conducting laboratory and location: [redacted]

Date of study initiation: April 13, 1992
GLP compliance: yes (x) no ( )
QA report: yes (x) no ( )
Drug Memantine HCl, **lot #** Groups 1-4: R 8825, Groups 5-7: R 7206, **radiolabel** Not applicable, **and % purity** Groups 1-4: 100.5%, Groups 5-7: 99.7%
Formulation/vehicle: Admixture of test article in standard diet [redacted]; samples taken for analysis of homogeneity from 3 levels in the feed bucket at start of study, at 7 days, and in week 13

Methods (unique aspects):
Dosing:

Species/strain: B6C3F1 Crl[redacted] Mice
#/sex/group or time point (main study): 10/sex/group in the main study, n=5/sex/group in the supplementary histopathology study
Satellite groups used for toxicokinetics or recovery: 6/sex/group for PK study (see Study Review under General Toxicology, above)
Age: Groups 1-4: 30-31 days (males), 41-42 days (females), Groups 5-7: 26-27 days (males), 34-35 days (females)
Weight: Groups 1-4: 15.1-18.9 g, Groups 5-7: 15.2-18.3 g
Doses in administered units: 0 (diet only, Groups 1 and 5), 5 (Group 2), 20 (Group 3), 80 (Group 4), 160 (Group 6), 320 (Group 7) mg/kg/day; the doses were selected based on the results of a 14-day dose-range-finding study [redacted] No. 7195/92), a second feed-only

control group (Group 5) was evaluated with the 160 and 320 mg/kg/day groups (Groups 6 and 7)
Groups 4, 5, 6, and 7 were selected for this histopathology study (n=5/sex/group)
Route, form, volume, and infusion rate: Oral in the diet

Observations and times: The standard toxicology parameters (e.g., clinical signs, body weights, clinical pathology) were evaluated for the main toxicology study (see under General Toxicology, above). For this study, the following evaluation was performed: Serial rostral and caudal sections were prepared from the retrosplenial and cingulate cortices, brain stem and the cerebellum. The sections were stained with H. & E. and embedded in paraffin for histological examination. The severity of abnormal histological findings were graded as follows: 1 = minimal, 2 = mild, 3 = moderate, 4 = marked.

Results: Minimal to moderate cytoplasmic vacuolization in the brain stem and cerebellum in 5/5 male mice and minimal neuronal necrosis in the cerebellum in 2/5 male mice at the high dose of 320 mg/kg/day, given daily for 13 weeks. Minimal vacuolization in the cingulate and retrosplenial cortices was observed with no difference in incidence in the control and treated mice.

Study title: Neurotoxic symptoms in female Sprague Dawley rats following acute application of the NMDA-antagonist memantine-HCl

Key study findings:

- Dose related increase in severity and incidence of vacuolation in the layers III and IV of the retrosplenial and cingulate cortices in rats administered memantine HCl by single IP injection at doses of 25 and 50 mg/kg, when examined by light and electron microscopy at 6 hours after dosing, comparable to lesions observed by MK-801 at 5 mg/kg
- Dose-related increase in severity and incidence of neuronal necrosis in the retrosplenial and cingulate cortices by memantine at doses of 25 and 50 mg/kg IP, when examined by light and electron microscopy at 48 hours after dosing
- Results characteristic of those described by Olney et al. (1989) for other NMDA receptor antagonists
- NOEL for vacuolation and necrosis induced in the retrosplenial and cingulate cortices by IP memantine HCl in this study was 12.5 mg/kg

Study no: Not provided
Volume # 56, and page # 15
Conducting laboratory and location: ——

Date of study initiation: Report date August 9, 1995
GLP compliance: yes ( ) no (x)
QA reports: yes ( ) no (x)
Drug Memantine HCl, **lot #** R 8825, **radiolabel** Not applicable, **and % purity** Not provided
Formulation/vehicle: Test article dissolved in sterile physiological saline

Methods: Female Sprague Dawley rats, —— weights 270-325 g, n=4-6 females/dose memantine, 4-6/dose MK-801 [positive control article], 7 vehicle [negative control article]) each

received a single intraperitoneal injection (5 ml/kg) of memantine HCl (12.5, 25, and 50 mg/kg), MK-801 (2.5, 5, and 7.5 mg/kg), or vehicle control article. The rats were sacrificed under pentobarbital-Na-salt anesthesia (40-60 mg/kg) at 6 hours (to detect neuronal vacuolation), 24 hours (for detection of recovery from vacuolation), and 48 hours (for detection of neuronal necrosis) after dosing. The rats were perfused (intracardial) with paraformaldehyde and glutaraldehyde in cacodylate buffer, heads removed and immersed in fixation fluid (paraformaldehyde/glutaraldehyde solution) overnight. The brains were removed and retrosplenial and anterior and posterior cingulate cortices excised. The brain regions were prepared for light microscopic examination (sectioned, stained with H&E or toluidine blue) at 10 X 100 magnification for vacuolation and 200X magnification for necrosis; for electron microscopic examination brain sections fixed in 5% cacodylate-buffered glutaraldehyde, embedded in epoxy rexin, postfixed in 1% osmium-tetroxide, embedded in Epon 812, sectioned ultra-thin, and contrasted with uranyl acetate and lead citrate.

Results:

Light Microscopy: At 6 hours after dosing, dose-related (frequency and severity) neuronal vacuolation was observed in layers III and IV in the large multipolar and pyramidal cell cytoplasm in both the retrosplenial and cingulate cortices at the dose of 25 and 50 mg/kg IP memantine HCl. The NOAEL for neuronal vacuolation was 12.5 mg/kg IP. No vacuolation was observed at the 24-hour examination, in agreement with the findings of Olney, *et al.* for other NMDA receptor antagonists. The results of the examination conducted 48 hours after dosing showed dose-related (in severity and incidence) neuronal degeneration in the retrosplenial and cingulate layers III and IV, with condensed nuclei, eosinophilic plasma and dark neurons at the doses of 25 and 50 mg/kg IP, and shrunken, necrotic neurons with eosinophilic cytoplasm and pyknotic triangular nuclei at the 50 mg/kg dose. The percent necrotic neurons (1,000 cortical cells examined per section) were1.74%, 2.00%, 1.72%, 4.27%, and 8.40% in the vehicle control, 25 mg/kg memantine (48h), 50 mg/kg memantine (24h), 50 mg/kg/ memantine (48h), and 7.5 mg/kg MK-801 treated groups.

Electron Microscopy: At 6 hours after dosing, vacuolation with dilated mitochondria and rough endoplasmic reticulum splitting of the nuclear membrane, dilated Golgi-complex, and intracytoplasmic vacuolation in the cytoplasm of the neuronal processes were observed with a dose-related increase in severity at the doses of 25 and 50 mg/kg IP memantine HCl. The NOAEL for electron microscopic detection of vacuolation by memantine was 12.5 mg/kg IP. At 24 hours after dosing, dark shrunken cytoplasm and degeneration in the mitochondria were observed in several degenerating neurons in the memantine-treated cells of the retrosplenial and cingulate cortices. At 48 hours after dosing, the neurons observed at 25 and 50 mg/kg IP showed shrunken, electron dense cytoplasm with irregular, fragmented cell boundaries, tightly packed ribosomes, and degenerating cell organelles, indented nucleus with homogenous chromatin with the nucleolus, and swollen astroglial processes.

The positive control, MK-801-treated sections showed dose-related increases in vacuolation at 6 hours and necrosis at 48 hours after dosing in the light microscopy, and large perinuclear and cytoplasmic vacuolation at 6 hours and degeneration with dead neurons containing fragmented nuclear membrane, disrupted cell boundaries, and caryorrhectic and caryolytic nuclei, with swollen astroglia and presynaptic processes at 48 hours in the electron microscopy. There were

no abnormalities in the light microscopy of the vehicle control treated tissues, and dark neurons similar to those in the MK-801-treated and memantine-treated tissues.

The results of the histopathology examination of the retrosplenial and cingulate cortices are presented in the following table:

Vacuolation and Neuronal Necrosis in the Retrosplenial and Cingulate Cortices of Female Rats Administered Memantine HCl by IP Injection, MK-801, and Vehicle Control

Treatment	Number of Rats Affected	Mean Percent Necrosis*	Mean Severity*
Vacuolation			
Memantine 12.5 mg/kg	0/6	-	0
Memantine 25 mg/kg	2/6	-	1
Memantine 50 mg/kg	4/4	-	3.625
MK-801 2.5 mg/kg	5/6	-	2.600
MK-801 5.0 mg/kg	4/4	-	3.625
Vehicle control	0/7	-	0
Necrosis			
Memantine 25 mg/kg	6/6	2.00%	1.33
Memantine 50 mg/kg (24h)	6/6	1.72%	1.00
Memantine 50 mg/kg (48h)	7/7	4.27%	2.57
MK-801 7.5 mg/kg	8/8	8.40%	4.00
Vehicle Control	6/6	1.74%	1.17

*Necrosis in the Retrosplenial Cortex, Anterior Cingulate Cortex, Posterior Cingulate Cortex; Severity of vacuolation and necrosis rated as follows: 1=minimal, 2=low, 3=medium, 4=high

Study title: Memantine HCl Olney lesions acute and repeated oral dosing in male and female Sprague Dawley rats

Key study findings:

- Minimal to slight intracytoplasmic vacuolation at 6 hours after dosing in layers III and IV of the retrosplenial and cingulate cortices of male and female rats administered memantine HCl by oral gavage at the single dose of 100 mg/kg (49X the MRHD of 20 mg in a 60 kg patient on a BSA basis, NOEL 50 mg/kg PO, 24X the MRHD)
- Minimal intracytoplasmic vacuolation in the neurons of layers III and IV in the retrosplenial and cingulate cortices, at 6 hours after dosing, observable in sections stained with toluidine blue, but not in the sections stained with H&E in the males and females at the high dose of 100 mg/kg (NOEL 50 mg/kg dietary, 24X the MRHD)
- No vacuolation in the retrosplenial and cingulate cortices in male and female rats after repeated oral dosing by gavage and dietary for 14 days
- Minimal (1-2/section) red neurons (necrotic neurons with condensed or pycnotic nuclei, and eosinophilic plasma) in 2/4 high dose males dosed by gavage (50 mg/kg/day PO), 3/4 high dose males dosed by dietary intake, 2/4 females at 25 mg/kg/day (12X the MRHD, and 4/4 females at 50 mg/kg/day dosed by gavage, and in 3/4 females at 100 mg/kg/day dietary memantine HCl
- The NOEL for cytoplasmic vacuolation in the repeated dosing experiments was 50 mg/kg/day by gavage and 100 mg/kg/day dietary memantine in the males and females

- The NOEL for neuronal necrosis in the repeated dosing experiments was 25 mg/kg/day (12X the MRHD) in the males and 12.5 mg/kg/day (6X the MRHD) in the females by oral gavage, and 50 mg/kg/day in the males and females by dietary intake
- Female rats more sensitive to the neurotoxic effect of memantine than were the males after acute and repeated oral intubation (higher frequency of vacuolated neurons at 100 mg/kg and 50 mg/kg/day, (acute and repeated dose, respectively) and vacuolation at the mid-dose (25 mg/kg/day repeated dose) not seen in the males
- No differences between the males and females in the frequency and sensitivity to dose were observed by the dietary route
- MK-801 at 5 mg/kg IP induced slight to marked vacuolation in layers IIII and IV in the retrosplenial and cingulate cortices of both male and female rats

Study no: Not provided
Volume # 56, and page # 66
Conducting laboratory and location: Merz + Co. GmbH & Co., Frankfurt
Date of study initiation: September 23, 1996
GLP compliance: yes ( ) no (x)
QA reports: yes ( ) no (x)
Drug Memantine HCl, **lot #** Not provided, **radiolabel** Not applicable, **and % purity** Not provided
Formulation/vehicle: Application by intubation (acute and repeated): test article dissolved in physiological saline at 10 ml/kg; Application by diet (acute and repeated): test article admixture in pellet diet (daily dose/12 g food, enforced by starvation)

Methods: Male and female Sprague Dawley rats [redacted] weights approximately 300 g, n=2/sex negative controls, 4/sex/dose memantine, 1/sex/positive control [MK-801]) were used for this study. In Protocol No. 1, the rats received a single dose of negative control vehicle (10 ml/kg, oral gavage), memantine at 25 mg/kg, 50 mg/kg, and 100 mg/kg (by oral gavage), or MK-801 (5 mg/kg IP), and were sacrificed (under Nembutal anesthesia at 60 mg/kg IP with heparin at 100 IU IP), heads perfused, and brain tissue fixed with paraformaldehyde (10 g/l), glutaraldehyde 25% (40 ml/l) in cacodylic acid and Na-salt 34 and24 g/l and $CaCl_2$ (300 mg/l) at 6 hours after dosing. In Protocol No. 2, the rats were administered negative control vehicle or memantine at 12.5, 25, and 50 mg/kg by oral gavage (10 ml/kg) once daily for 14 days. The positive control group received MK-801 at 5 mg/kg IP 6 hours before sacrifice. The rats were sacrificed 6 hours after the last dose, and tissues prepared as described under Protocol No. 1. In the acute dietary arm (Protocol No. 3), the rats received a single dose of negative control (diet only, [redacted] standard diet for rats and mice, pelleted form), memantine HCl at 25, 50, and 100 mg/kg (mixed in diet), or MK-801 (5 mg/kg IP), were sacrificed six hours after dosing, heads perfused and brain tissue fixed as described under Protocol No. 1, above. In Protocol No. 4, the rats were administered negative control (diet alone as under Protocol III) or memantine HCl at 25, 50, and 100 mg/kg/day (mixed in diet, once daily for 14 days) or MK-801 (5 mg/kg IP, once, 6 hours before sacrifice). The rats were sacrificed 6 hours after the last dose, heads perfused and brain tissue fixed as described under Protocol No. 1, above.

The retrosplenial and anterior cingulate cortices were isolated, and half the specimens were embedded in Epon, post-fixed with 1% osmium tetroxide, sectioned, stained with Toluidine blue, and the other half of the specimens were embedded in paraffin, sectioned (semi-thin) and stained with Haemalaun and Eosin, for examination of Layers III and IV by light microscopy.

Vacuolated neurons were graded as minimal (+, 1-3 vacuolated neurons, 1-2 red neurons per section), slight (++, 3-6 vacuolated neurons with 2-4 red neurons per section), moderate (+++, 6-12 vacuolated neurons with 4-10 red neurons per section) and marked (++++, 12 or more vacuolated neurons with 10 or more red neurons per section).

Results: The results of the light microscopy examinations are presented in the following tables:

Olney-Vacuolation in the Retrosplenial and Cingulate Cortices of Rats Administered Memantine HCl in a Single Dose by Oral Gavage and in the Diet*

Dose (mg/kg)	Animal Number		Olney-Vacuolation					
			Retrosplenial Cortex (H&E)		Cingulate Cortex (H&E)		Semi/Toluidine Blue	
Acute Oral Intubation								
	Males	Females	Males	Females	Males	Females	Males	Females
0	1	101	-	-	-	-	-	-
	2	102	-	-	-	-	-	-
	3	103	-	-	-	-	-	-
	4	104	-	-	-	-	-	-
Memantine 25	5	105	-	-	-	-	-	-
	6	106	-	-	-	-	-	-
	7	107	-	-	-	-	-	-
Memantine 50	8	108	-	-	-	-	-	-
	9	109	-	-	-	-	-	-
	10	110	-	-	-	-	-	-
	11	11	-	+	+	+	+	-
Memantine 100	12	112	-	+	++	++	++	+
	13	113	+	++	++	++	+	+
	14	114	-	+	-	++	-	-
MK-801 5	15	115	++	++++	++	+++	++	++++
Acute Oral Dosing by Diet								
0	1	101	-	-	-	-	-	-
	2	102	-	-	-	-	-	-
	3	103	-	-	-	-	-	-
	4	104	-	-	-	-	-	-
Memantine 25	5	105	-	-	-	-	-	-
	6	106	-	-	-	-	-	-
	7	107	-	-	-	-	-	-
Memantine 50	8	108	-	-	-	-	-	-
	9	109	-	-	-	-	-	-
	10	110	-	-	-	-	-	-
	11	11	-	-	-	-	-	+
Memantine 100	12	112	-	-	-	-	+	-
	13	113	-	-	-	-	-	+
	14	114	-	-	-	-	+	-
MK-801 5	15	115	++	+++	+	+++	++	-

*See Grading under Methods, above

Olney-Vacuolation in the Retrosplenial and Cingulate Cortices of Rats Administered Memantine HCl for 14 Days by Oral Gavage and in the Diet*

Dose (mg/kg/day)	Animal Number		Red Neurons (H&E/Semi)						Vacuoles (H&E.Semi)	
			Retrosplenial Cortex		Cingulate Cortex		Tol.			
	Males	Females	Males	Females	Males	Females	Males	Females	Males	Females

14-Day Repeated Oral Intubation										
0	1	101	-	-	-	-	-	-	-	-
	2		-	-	-	-	-	-	-	-
Memantine 12.5	3	103	-	-	-	-	-	-	-	-
	4	104	-	-	-	-	-	-	-	-
	5	105	-	-	-	-	-	-	-	-
	6	106	-	-	-	-	-	-	-	-
Memantine 25	7	107	-	-	-	-	-	-	-	-
	8	108	-	-	-	+	-	+	-	-
	9	109	-	-	-	+	-	+	-	-
	10	110	-	-	-	-	-	-	-	-
Memantine 50	11	111	-	+	-	+	-	+	-	-
	12	112	-	+	-	-	-	+	-	-
	13	113	+	-	+	+	-	-	-	-
	14	114	-	+	-	-	-	-	-	-
MK-801 5	15	115	-	-	-	-	-	-	+++	+++
		102		-		-		-		++
14-Day Oral Application by Diet										
0	1	101	-	-	-	-	-	-	-	-
	2	102	-	-	-	-	-	-	-	-
Memantine 25	3	103	-	-	-	-	-	-	-	-
	4	104	-	-	-	-	-	-	-	-
	5	105	-	-	-	-	-	-	-	-
	6	106	-	-	-	-	-	-	-	-
Memantine 50	7	107	-	-	-	-	-	-	-	-
	9	108	-	-	-	-	-	-	-	-
	9	109	-	-	-	-	-	-	-	-
	10	110	-	-	-	-	-	-	-	-
Memantine 100	11	111	-	-	+	+	+	-	-	-
	12	112	-	-	+	++	-	+	-	-
	13	113	+	-	+	+	-	-	-	-
	14	114	-	-	-	-	-	-	-	+
MK-801 5	15	115							+++	++++

Study titles:

Neuronal vacuolation and necrosis after continuous infusion of the NMDA-receptor antagonist memantine-HCl in the rat

Memantine – Determination of Memantine in Plasma of Rats

Key study findings:

- Dose-related increase in severity and frequency of intracytoplasmic vacuolation after 6 hours continuous infusion at 7.82 (2.23% neurons lesioned, minimal to mild) and 15.65 (5.25% neurons lesioned, moderate to marked severity) mg/kg/h
- Dose-related increase in severity and frequency of neuronal necrosis at 72 hours after an 18-hour infusion at 3.14 (1.67% neurons lesioned, mild to moderate severity) and 6.28 (5.82% neurons lesioned, moderate to marked severity)
- Distribution pattern of necrosis similar to that for vacuolation
- Vacuolation and necrosis observed in large multipolar and pyramidal neurons of layers III and IV in the retrosplenial and cingulate cortices

- Necrosis characterized by shrunken neurons with eosinophilic cytoplasm and pyknotic/triangular nuclei
- NOEL for memantine-induced vacuolation and necrosis in the retrosplenial and cingulate cortices in rats not identified (<3.14 mg/kg IV)
- Lowest dose associated with vacuolation (7.82 mg/kg/h) corresponded to a mean plasma memantine concentration of 2508-2633 ng/ml (31X-33X the steady state plasma level of 80 ng/ml at the therapeutic dose of 20 mg/day in humans)

Study no: 9258/1/95 and ZA045-95
Volume # 56 and #57, **and page #** 88 and #148
Conducting laboratory and location: Toxicokinetic study by Merz + Co. GmbH & Co.,
Date of study initiation: October 13, 1995, November 15, 1995
GLP compliance: yes (x) no ( )
QA reports: yes (x) no ( )
Drug Memantine HCl, **lot #** R 8825, **radiolabel** Not applicable, **and % purity** 99.7%
Formulation/vehicle: Test article dissolved in 0.9% NaCl solution

Methods: Female Sprague Dawley rats (Interfauna, weights 265-342 g, n=4/group in the negative and positive control groups, n=7/dose in the memantine treated groups) were used in this study. Each rat was administered control vehicle or memantine at doses of 3.14, 6.28, 7.82, and 15.65 mg/kg (2 ml/kg/h, total doses 57, 113, 47, and 94 mg/kg, respectively) by continuous intravenous infusion (by indwelling cannula in the jugular vein) over 6 hours (0, 7.82 and 15.65 mg/kg) and 18 hours (0, 3.14, and 6.28 mg/kg). The positive control animals received a single intraperitoneal injection of MK-801 at 5 mg/kg. At the end of the 6- and 18-hour vehicle and memantine infusions, and 6 and 18 hours after the MK-801 injection, blood (1-2 ml) was withdrawn from the postorbital venous plexus under nembutal (40 mg/kg, with heparin 100 IU) anesthesia for determination of plasma drug concentrations. The rats were perfused with paraformaldehyde/glutaraldehyde (4%/4%) solution in cacodylate buffer at 6 or 72 hours after the start of infusion. The heads were removed and placed in fixation fluid, and sent to the , for processing. The brains were removed, and retrosplenial and anterior and posterior cingulate cortices were isolated. The isolated regions were embedded in paraffin, sectioned and stained with Haemalaun and Eosin for histological examination at 10 x 20 magnification. Approximately 97603 neurons per section were examined. Additional slices were saved for later electron microscopy.

Results: The results of the microscopic examination are presented in the following table:

Lesions in the Retrosplenial and Cingulate Cortices of Rats Administered Memantine HCl by Continuous Intravenous Infusion

Treatment	Dose (mg/kg)	Infusion Time (h)	Evaluation Time (h after start of infusion)	Percent Lesioned Neurons
NaCl Vehicle	0 (5 ml/kg/h IV)	6h 18h	6h 72h	0% 0%
MK-801	5 mg/kg IP	-	6h 72h	6.15% (marked vacuolation in cingulate) 5.30% (necrosis in cingulate)

Memantine HCl	7.82 mg/kg/h IV	6h	6h	2.23% (minimal to mild vacuolation in cingulate)
Memantine HCl	15.65 mg/kg/h IV	6h	6h	5.25% (moderate to marked vacuolation in cingulate)
Memantine HCl	3.14 mg/kg/h IV	18h	72h	1.67% (mild to moderate necrosis in cingulate and retrosplenial cortices)
Memantine HCl	6.28 mg/kg/h IV	18h	72h	5.82% (moderate to marked necrosis in retrosplenial and cingulate cortices)

There was a dose-related increase in severity of neuronal toxicity (vacuolation at 6 hours and necrosis observed at 72 hours after the start of intravenous infusion). Necrosis observed at 72 hours showed the same distribution pattern as the vacuolation pattern observed in the 6-hour examinations. Both vacuolation and necrosis were observed in the large multipolar and pyramidal neurons of layers III and IV in the retrosplenial and cingulate cortices. The necrosis was characterized by shrunken neurons with eosinophilic cytoplasm and pyknotic/triangular nuclei.

The results of the plasma memantine measurements are presented in the following table:

Plasma Memantine Concentrations in Rats Administered Memantine HCl by Continuous Intravenous Infusion

Rat Number	Dose Memantine (mg/kg)	Infusion Duration (h)	Plasma Memantine Concentration (ng/ml)*	Second Measurement: Plasma Memantine Concentration (ng/ml)*
1	0 (0.9% NaCl)	6h	0	Not done
10		6h	0	
14		18h	0	
21		18h	0	
11	7.82 mg/kg/h	6h		
12				
15				
33				
34				
35				
36				
2	15.65 mg/kg	6h		
3				
4				
5				
6				
17	3.14 mg/kg	18h		Not done
18				
19				
20				
22				
23				
24				
25	6.28 mg/kg	18h		Not done
26				
27				
28				
29				

31			Llq	

*Llq = Lower limit of quantitation

Study title: Memantine-HCl Toxicokinetic Study in Baboons by Repeated Oral Administration for 14 Days: Includes Study Report entitled: "Memantine and metabolites: Memantine HCl toxicokinetic study in baboons (plasma and urine) by repeated oral administration for 14 days"

Key study findings:

- No evidence of neuronal vacuolation and necrosis in Layers III and IV of the cingulate cortices of baboons administered memantine HCl by oral gavage at 8 mg/kg/day daily for 14 days; retrosplenial cortices not examined
- Mean plasma $AUC_{0\text{-}inf}$ values of 2020 ng.h/ml and 3890 ng.h/ml on dosing Days 1 and 12, respectively
- Dose studied (8 mg/kg/day) represented approximately 13X the MRHD of 20 mg in a 60 kg patient on a BSA basis and 1X-2X the MRHD on an AUC basis

Study no: PTX 52/932357 (Neurotoxicity study); Merz study No. ZA090-93/PTX52 (TK study)
Volume # 56, **and page #** 160 (Neurotoxicity Study); **Volume #** 58, **page #**1 (TK Study)
Conducting laboratory and location:
Department of Biolog. Analytics, Merz + Co. GmbH & Co.
Date of study initiation: September 16, 1993
GLP compliance: yes (x) no ( )
QA report: yes (x) no ( )
Drug Memantine HCl, **lot #** R 8825, **radiolabel** Not applicable, **and % purity** 100%,
Formulation/vehicle: Test article dissolved in distilled water (0.2% w/v), formulation confirmed by analysis of samples before the first dose and at 1 week

Methods (unique aspects):
Dosing:
Species/strain: baboons
#/sex/group or time point (main study): 2/sex/group
Satellite groups used for toxicokinetics or recovery: None
Age: 1.5-2.5 years
Weight: 3.5-4.4 kg
Doses in administered units: 8 mg/kg/day
Route, form, volume, and infusion rate: Oral by gastric intubation at 4 ml/kg, once daily for 14 days

Observations and times:
Clinical signs: 3X daily
Body Weights: Weekly before dosing, 2X weekly during dosing
Food consumption: Daily
Toxicokinetic Sampling: Baseline and on Dosing Days 1 and 12 at 0, ½, 1, 1 ½, 2, 4, 6, 8, 12, and 24 hours after dosing
Urine Samples: Baseline and on Dosing Days 10-11, and 14-15 for 24-hour periods each

Terminal Studies: Histopathology examination of the brain: 6 hours after the last dose, the animals were sacrificed under ketamine and pentobarbitone sodium anesthesia, perfused (intracardial) with 4% formaldehyde in phosphate buffered saline. The brains were removed and divided. The cortex, hippocampus, striatum, brain stem and other areas were isolated from the left side, for analysis of memantine HCl and metabolite concentrations. The anterior, posterior, and isthmus of the posterior cingulate cortices were isolated from the right side of the brains, fixed in buffered formalin, embedded in paraffin, sectioned (4 mcm) and stained with H&E for microscopic examination.

Results:

Mortality: No deaths

Clinical signs: Drooping eyelids at 1 ½ hours after dosing, duration 2-4 hours

Body Weights: No treatment-related effects

Food consumption: No treatment-related effects

Toxicokinetic Sampling: The results of the plasma memantine analyses are presented in the following table:

Results of the Toxicokinetic Analysis in Baboons Administered Memantine HCl at 8 mg/kg/day by Oral Gavage Daily for 14 Days (n=4)

Parameter	Maximum	Median	Minimum	Mean (± S.D.)
		Day 1		
AUC_{0-t} (ng.h/ml)		1880.0		1860.0 ± 304.0
AUC_{0-inf} (ng.h/ml)		2020.0		2020.0 ± 257.0
Cmax (ng/ml)		217.00		218.00 ± 82.30
Tmax (h)		2.00		2.25 ± 1.26
T1/2 (h)		6.64		6.44 ± 1.30
Vd (l)		36.2		37.7 ± 11.5
Cl (ml/min)		66.20		66.80 ± 8.56
MRT (h)		9.64		9.93 ± 2.23
		Day 12		
AUC_{0-t} (ng.h/ml)		3360.0		3630.0 ± 789.0
AUC_{0-inf} (ng.h/ml)		3540.0		3890.0 ± 961.0
Cmax (ng/ml)		394.00		397.00 ± 99.60
Tmax (h)		2.50		2.50 ± 1.73
T1/2 (h)		5.31		5.73 ± 1.03
Vd (l)		17.2		17.2 ± 1.3
Cl (ml/min)		37.90		35.70 ± 7.52
MRT (h)		9.00		8.82 ± 1.06

Urine Samples: The urine concentrations of memantine and memantine metabolites are presented in the following table:

24-Hour Urine Memantine and Memantine Metabolite Concentrations in Baboons Administered Memantine HCl at 8 mg/kg/day by Oral Gavage for 14 Days

Component	Animal 74f2 (female)	Animal 744 (female)
MRZ 2/145 (mg)	1.89	1.14
MRZ 2/371 (mg)	0.98	1.00
MRZ 2/373 (mg)	0.50	0.69
MRZ 2/374 (mg)	0.79	0.81
Excretion (mg)	4.16	3.64

Excretion (% of dose)	17.99	12.99

Terminal Studies: No evidence of neuronal vacuolation and necrosis in layers III and IV of the cingulate cortex; however, the study is considered to be less than optimal because the dose was below the MTD based on absence of toxicity and represented only 1X-2X the MRHD of 20 mg/day in a 60 kg patient on an AUC basis, the study lacked concurrent controls, and the retrosplenial cortices were not examined.

IX. DETAILED CONCLUSIONS AND RECOMMENDATIONS:

Overall Summary and Evaluation:

Introduction: Memantine is an uncompetetive N-methyl-D-aspartate (NMDA) receptor antagonist with moderate affinity, with strong voltage-dependent characteristics and rapid channel unblocking kinetics. The rationale for the development of this drug product for the treatment of moderate to severe Alzheimer's dementia is based on the hypothesis that excessive neuronal calcium influx induced by overstimulation of the NMDA receptor during prolonged endogenous glutamate transmission is responsible in part for the pathophysiology and neuronal cell death associated with neurodegenerative central nervous system disorders, such as Alzheimer's disease, and that by decreasing excessive glutamate transmission, memantine will attenuate excitotoxic neuronal destruction and improve cognitive function in patients with severe Alzheimer's disease. Memantine is approved and marketed in 41 countries, under the tradenames Akatinol®, Axura®, and Ebixa®. The currently marketed therapeutic dose and proposed dose in this submission is 20 mg/day. Fifty-three clinical trials were conducted in healthy volunteers and dementia patients, with 21 studies in 2625 patients ongoing at the time of the NDA submission. The clinical safety data indicate that memantine, at therapeutic doses of up to 20 mg/day, is well tolerated and produces minor dose-related adverse effects, including dizziness, headache, confusion, and constipation.

For this submission, *in vitro* and *in vivo* studies were conducted to evaluate the mechanism of action of memantine, and to support the proposed therapeutic indication. Safety pharmacology studies were conducted in mice, rats, and dogs, to include evaluation of memantine effects on central nervous system (CNS), cardiovascular, respiratory, gastrointestinal and renal function. Pharmacokinetic and toxicokinetic analyses were performed in mice, rats, rabbits, dogs, and baboons, and included characterization of oral bioavailability and ADME parameters. The sponsor submitted the results of acute and repeated dose oral toxicology studies for dosing durations of up to 52 weeks in rats, dogs, and baboons. Ocular toxicity was investigated in albino and pigmented rats and in dogs, because previous studies showed affinity of memantine for melanin *in vitro* and in whole body autoradiography in pigmented rats, high memantine content in and slow release from pigmented tissues and eyes in several species, amphiphilic nature of memantine, accumulation of memantine in lysosomes which is associated with retinal damage by other drugs, and corneal lesions and focal lens turbidities in multiple dose studies in rats. Special neurotoxicity studies were conducted in mice, rats, and monkeys, to evaluate the potential of memantine to induce NMDA-receptor antagonist-induced intracytoplasmic vacuolation in the retrosplenial and cingulate cortices. Additionally, the sponsor submitted the final study reports on a Standard Battery of studies to evaluate the genotoxic potential of

memantine, and the results of studies on potential carcinogenic and reproductive effects of memantine.

Pharmacology: Patch clamp studies on memantine and memantine metabolites demonstrated that memantine is a voltage-dependent, low affinity, uncompetetive (open channel) NMDA receptor antagonist. The metabolite MRZ 2/169 showed high potency, but the metabolites MRZ 2/371, MRZ 2/373, MRZ 2/374, and MRZ 2/375 were very weak antagonists at the NMDA receptor. MRZ 2/169 is excreted in the urine of dogs at approximately 5% total radioactivity, and is not detected in mice, rats, baboons, and humans. The memantine metabolite MRZ 2/325 (dimethyl-gludantan) was a very weak antagonist at four NMDA receptor subtypes in Xenopus oocytes, and is therefore unlikely to be involved in memantine pharmacological effects in animals and humans.

In studies on drug activity related to the proposed indication, memantine increased locomotor activity in mice and rats, shortened the time to appearance of spontaneous movements in mice after head blow (return to consciousness in animal model of head injury), decreased the number of trials in a retention session in anoxia-treated mice, increased learning (avoidance rate) in rats with internal capsule lesions, increased hippocampal alpha power and maintained cortical beta2 power in freely moving rats, increased beta2 power and decreased alpha1 and apha2 power in spontaneous cortical EEGs in cats, increased total power of rhythmic slow wave activity in the hippocampus after intraseptal administration in rats, antagonized the disturbed EEG patterns in pons ischemic rats, and increased DOPAC and HVA levels, but not the noradrenaline, MOPEG, dopamine, serotonin, and 5-HIAA levels in the nucleus accumbens in rats. Memantine had no effect on decreased locomotor activity in the retention session of a habituation assay, and the latency to enter the dark compartment at a retention session in cycloheximide-induced amnesia in mice. It appears that memantine stimulates locomotor activity via dopamine release from the nucleus accumbens and enhanced memory tasks in animals by activation of hippocampal function via dopamine release in the septum and nucleus accumbens.

Neuroprotective effects of memantine were observed in rats injected with beta-amyloid 1-40 (AB1-40) to induce hippocampal neuronal degeneration, a model of degeneration observed in Alzheimer's disease patients. Specifically, memantine administered subcutaneously by osmotic pump at 30 mg/ml for 9 days, resulting in steady state plasma concentrations of 1.40-3.58 mcM, decreased the extent of AB1-40 induced neuronal loss in the CA1 subfield, reduced the number of MAP2 positive somata indicating cytoskeletal alterations in several hippocampal areas, reduced the immunoreactivity for astroglial and microglial macrophage markers GFAP and ED1, and reduced apoptotic profiles in the hippocampus 7 days after AB1-40 injection.

Safety Pharmacology: Neurological Effects: Memantine HCl had no effect on neuropharmacological parameters in the IRWIN test at the dose of 10 mg/kg by oral gavage in mice. The parameters included indices of awareness (alertness, visual placing, passivity, and stereotypy), mood (grooming, vocalization, restlessness, and aggression), motor activity (reactivity, spontaneous activity, touch response, and pain response), CNS excitation (startle response, Straub tail, tremors, twitches, and convulsions), posture (body posture, limb position, staggering gait, abnormal gait, and righting reflex), muscle tone (limb tone, grip strength, body sag, body tone, and abdominal tone), reflexes (pinna, corneal, and ipsilateral flexor reflex), and autonomic function (writhing, pupil size, palpebral opening, exophthalmos, urination, salivation, piloerection, hypothermia, skin color, and respiration rate). At 30 mg/kg, there was decreased

awareness (ability to place self after being put in different positions, and decreased righting reflex). Most neuropharmacological parameters were affected by memantine at the high dose of 100 mg/kg PO, including decreased awareness, motor activity, CNS excitation, muscle tone, reflexes, and autonomic function (respiratory rate). In another study, memantine increased spontaneous motility in mice at oral doses of 5-80 mg/kg in a dose-related manner, but the effects were 15X-20X less than those by d-amphetamine at 1-2.5 mg/kg. There was a dose-related increase in sleeping time in male and female mice administered oral memantine at doses of 15, 30 (50% increase) and 60 mg/kg (100%).

Potential anticonvulsive properties of memantine HCl were investigated in mice given doses of 10-33 mg/kg orally. Electroshock-induced convulsions, 60 minutes after dosing, were decreased significantly, by 0, 40%, 60%, and 100% at 10, 15, 22, and 33 mg/kg memantine (ED50 = 18.4 mg/kg PO), respectively. In comparison, diazepam provided 80% protection from the tonoclonic convulsions induced by electroshock in the mice at 20 mg/kg PO.

Oral memantine HCl increased the number of pentetrazol-induced convulsions by 167% at 22 mg/kg PO and 256% at 33 mg/kg PO in mice, and induced convulsions in 10%, 80%, and 100% mice at the doses of 10, 30, and 100 mg/kg PO (ED50 17.8 mg/kg PO), respectively, when given 60 minutes prior to subthreshold doses of pentetrazol (50 mg/kg SC). The positive control article, d-amphetamine (50 mg/kg) induced convulsions in 40% mice given the subconvulsive dose of pentetrazole. Memantine HCl had no pro-convulsive effects when given at doses of 10-100 mg/kg PO 60 minutes prior to subthreshold electrical current in mice.

In a safety pharmacology study on the potential effects of memantine HCl on body temperature in mice, memantine antagonized reserpine-induced reduction in body temperature at all doses tested (10-100 mg/kg PO) at 4 hours after reserpine treatment, without a dose-relationship.

Safety Pharmacology: Cardiovascular Effects: Memantine had minor effects on HERG channel function, indicated by approximately 29% reduction in the fluorescence assay and 13% reduction when measured by electrophysiology. Memantine and MRZ 2/579 reduced the HERG inward tail current amplitude approximately 13% at the highest concentrations tested of 100 mcM. In comparison, amantadine reduced the current by 27% at 500 mcM, MRZ 2/705 by 52% at 100 mcM, and budipine by 100% at 100 mcM.

Memantine HCl given by duodenal gavage in anesthetized beagle dogs induced a dose-related decrease in cardiac minute output at 10 mg/kg (7.5%) and 30 mg/kg (20%), and stroke volume at 10 mg/kg (14%) and 30 mg/kg (32%) when compared to the vehicle control values at 10 minutes after dosing. Systolic left ventricular blood pressure was decreased by intraduodenal memantine at 30 mg/kg when compared to vehicle control, at 15 (9%) and 30 (18%) minutes after dosing.

Safety Pharmacology: Renal Effects: In a study on renal effects in rats, memantine HCl administered at doses of 10-40 mg/kg by oral gavage increased urine volume significantly during the period from 2-5 hours after dosing at the highest dose tested, but there were no differences in total urine volume from control volume in the memantine-treated rats for the 24-hour period after dosing. The memantine-induced increase in urine volume was highest at 2 hours (48% higher than control volume), and greater than the increase in urine volume induced by the positive control article, furosamide (30% increase over control). There was a dose-related increase in sodium and chloride excretion in the rats, at all doses from 10-40 mg/kg PO (5X-19X the MRHD

of 20 mg in a 60 kg patient on a BSA basis) during the period from 2-5 hours after dosing, but no differences from control values overall for the 24-hour period after dosing. The greatest increases in sodium and chloride excretion were observed in the second hour after drug administration. Sodium excretion was increased 126%, 188%-241%, and 205%-276% at 10, 20 and 40 mg/kg oral memantine, respectively, compared to 232%-403% in the furosamide-treated rats during the 5-hour period after dosing. Chloride excretion was increased 124%-184% and 120%-197% at 20 and 40 mg/kg oral memantine, respectively, compared to 293%-637% in the furosamide-treated rats during the 5-hour period after dosing.

Safety Pharmacology: Gastrointestinal Effects: There was a dose-related inhibition of intestinal motility by memantine HCl (0%, 40%, 50%, and 60% animals showed no charcoal in the cecum at 0, 10, 20, and 40 mg/kg PO, ED50 20 mg/kg PO) measured 4 hours after dosing in female rats administered memantine HCl by gastric intubation.

Memantine HCl induced a slight spasmogenic effect in isolated guinea-pig ileum at the concentration of $1X10^{-5}$ g/ml (slight), and a greater effect at $1X10^{-4}$ g/ml (26% effect caused by acetylcholine) and $1X10^{-3}$ g/ml (9% effect caused by acetylcholine). The agonist effect of memantine was not antagonized by papaverine ($3X10^{-5}$ g/ml), antazoline ($3X10^{-8}$ g/ml), and atropine ($3X10^{-8}$ g/ml). Memantine also showed concentration-related spasmolytic (antagonist) effects at $1X10^{-5}$ g/ml, with complete antagonism of agonist-induced (acetylcholine ($5X10^{-7}$ g/ml), histamine ($5X10^{-8}$ g/ml), barium chloride ($2X10^{-4}$ g/ml), and 5-hydroxy-tryptamine ($1.5X10^{-8}$ g/ml)) contractions at $1X10^{-4}$ g/ml.

Safety Pharmacology: Abuse Liability: In an intravenous (IV) self-administration assay, morphine-dependent, but not morphine-naïve mice self-administered memantine. Memantine attenuated the severity of morphine withdrawal in the morphine-dependent mice. Intraperitoneal (IP) memantine injections produced significant conditioned place preference at the dose of 30 mg/kg, but not at lower doses, in adult male Wistar rats. However, in another study, repeated IP memantine injections at up to 30 mg/kg did not induce conditioned motor activity after repeated memantine-environment pairings. Although memantine partially substituted for PCP in drug discrimination studies in rats and monkeys, the rates of responding were significantly lower than for PCP, except at high doses.

Pharmacokinetics/Toxicokinetics: Memantine pharmacokinetics were studied in rats, dogs, and baboons. In female Sprague Dawley rats administered memantine HCl at 12.5-50 mg/kg IP, the increases in peak plasma levels and exposure (AUC values) were greater than linear. The AUC increased approximately 2.5X when the memantine dose was doubled. Peak plasma levels were observed at approximately 0.5-1 hour after dosing (Tmax), and the half-life was 3-4.5 hours. Clearance was rapid (22.4-38.4 ml/min) and the large volume of distribution (8.7-10.6 L) suggested extensive tissue distribution. After oral dosing (25-100 mg/kg) in male and female rats, there was a linear increase in peak plasma concentration (Cmax) with dose and 2.5X-3.1X increase in exposure (AUC) values as the dose was doubled. The Cmax was higher and AUC considerably higher in the female rats than in the male rats. The peak plasma concentrations were observed at 0.5 hours across doses, and half-life of elimination increased slightly with dose from 5.7 h to 7-9.4 hours at 25 and 50-100 mg/kg memantine. Clearance decreased with dose. The high volume of distribution values (14-27 L) suggested extensive tissue distribution.

In baboons administered memantine at the dose of 8 mg/kg/day in the diet for 12 consecutive days, the peak plasma levels (Cmax) and exposure (AUC) increased 100% on Day 12 (397 ng/ml and 3600-3900 ng.h/ml, respectively) compared to the Cmax and AUC levels observed on Day 1. There was no change in Tmax (approximately 2.25-2.50 h), and the half-life was slightly decreased with repeated dosing from 6.44 hours on Day 1 to 5.73 hours on Day 12. The volume of distribution (38 L on Day 1 and 17 L on Day 12) suggested extensive tissue distribution. Clearance was rapid, at 67 and 36 ml/min on Days 1 and 12, respectively.

A comparative pharmacokinetics study showed lower elimination half-lives in dogs and baboons (between 4.4 and 10.2 hours) than in humans (52-58 hours) after oral administration of memantine. The concentration of ^{14}C-memantine was higher in whole blood than in plasma in the humans, whereas in baboons and dogs, the concentrations were higher in plasma than in blood.

Memantine was rapidly absorbed by the oral route, producing peak plasma levels within ½-2 hours after dosing in animals. The bioavailability was approximately 80% in rats, regardless of dose.

Distribution was predominantly to the kidneys, liver, lungs, testes, and intestinal fat, with lower concentrations found in brain, muscle, lacrimal gland and blood in rats. The tissue concentrations were generally increased 2X-2.5X after repeated dosing when compared to tissue levels after administration of single doses. In the eyeball, the highest concentrations of memantine were found in the cornea and sclera/choroid/retina, with lower concentrations in the lens and vitreous body. Comparative studies in albino and pigmented rats showed rapid absorption and distribution to body tissues, with maximum concentrations at 1-6 hours after dosing in both strains, except for peak uveal tract and pigmented skin concentrations at 6-12 hours in the pigmented rats. The highest concentrations were found in the gastrointestinal tract, kidneys, urinary tract, liver, adrenal, lachrymal glands, Harderian gland, salivary glands and spleen in both strains, and in the eye and particularly in the uvea (vascular layer of the eyeball) in the pigmented rats. Higher concentrations of radioactivity were found in the eyes of the pigmented rats than in the albino rats' eyes, that could be detected at trace levels 28 days after drug administration. In the rabbit, maximal tissue concentrations were highest at 30 minutes after intravenous dosing, in the lungs, kidneys, ovaries, brain and liver. Memantine crossed the blood brain barrier and placenta. Fetal memantine concentrations were highest at 30 minutes after dosing, and similar to maternal levels in fetal blood and liver at that timepoint. In baboons administered 5 mg/kg ^{14}C-memantine HCl b.i.d. for 7 days, the highest tissue concentrations in baboons were observed, in decreasing order of concentration, in the bile, colon contents, kidneys, liver, lungs, medulla oblongata, spleen, lymph nodes, gonads, spinal cord, stomach contents, gyrum prae- and postcentralis, thalamus, and cingulum at 24 hours after the last dose. By 96 hours after the last dose, the highest concentrations were found in the bile, lungs, kidneys, adrenals, skin, spleen, liver, and colon contents.

An *in vitro* study was conducted to evaluate melanin binding of ^{14}C-memantine (0.06-180 mcmol/7ml) in a pigment suspension. The results showed 41.44%, 19.75%, and 10.68% drug bound to melanin at the concentrations of 0.01277, 0.1277, and 0.5319 mcmol/mg melanin. Insignificant binding to melanin was found at 12.77 and 38.30 mcmol/mg melanin. No glucose binding was observed. In conclusion, memantine bound to melanin moderately at low concentrations that are expected to be found at therapeutic doses.

The major metabolites identified in mouse urine were MRZ 2/373 (3-hydroxymethyl-metabolite, 14.5% relative to memantine content), MRZ 2/374 (4/8-hydroxy-regio-isomers, 8.5%), and total MRZ 2/525 (free and conjugated N-hydroxy-metabolite, 6.6%). Minor metabolites were analogues of MRZ 2/564 (1-amino-2-hydroxy-3,5-dimethyl-adamantane HCl, 2 isomers, 5.6%) and MRZ 2/677 (1-amino-2-hydroxy-5,7-dimethyl-adamantane HCl) or (1-amino-3,5-dimethyl-9-hydroxy-adamantane HCl, 5.6%). The major metabolites in rat urine were MRZ 2/373 (1-amino-3-hydroxymethyl-5-methyl-adamantane HCl, 294%), MRZ 2/375 (1-amino-3-carboxy-5-methyl-adamantane HCl, 54%), MRZ 2/374 (isomeric mixture of 1-amino-3,5-dimethyl-4/8-hydroxy-adamantane HCl, 48%), and MRZ 2/325 (1-N-(3.5-dimethyl)-gludantan, 18.6%). The major metabolites in baboon urine were MRZ 2/529 (II, 1-nitro-7-hydroxy-3,5-dimethyl-adamantane, 505.6%), MRZ 2/374 (4/8-hydroxy-regio-isomers, 188%), MRZ 2/373 (3-hydroxymethyl-metabolite, 119%), MRZ 2/371 (1-amino-3,5-dimethyl-7-hydroxy-adamantane HCl, 118%), MRZ 2/529 (1-nitro-7-hydroxy-3,5-dimethyl-adamantane, 67%), and MRZ 2/524 (45%). The major metabolites in the human urine were MRZ 2/325 (1-N-(3.5-dimethyl)-gludantan, 19%), MRZ 2/374 (4/8-hydroxy-regio-isomers, 18%), MRZ 2/524 (13%), and MRZ 2/525 (total N-hydroxy-memantine, 3%). Re-evaluation of the N-nitroso-deaminated (MRZ 2/524) and 1-nitro-deaminated (MRZ 2/523) metabolites in the samples from a study using acidic extraction showed lower concentrations of MRZ 2/524 in mouse (9363 ng/ml), rat (499.5 and 1645 ng/ml in 2 samples), baboon (3968 ng/ml), and human (380 ng/ml) urine. The results showed lower concentrations of MRZ 2/523 in mouse (289 ng/ml), rat (132 and 51 ng/ml), baboon (106 ng/ml), and human (5 ng/ml) urine.

MRZ 2/374 was found in high concentration in all 5 species including humans, MRZ 2/373 was found in all nonhuman species and at a lower concentration in humans, and MRZ 2/375 was found in the rodent (mouse and rat) urine but not detected in human urine. Overall, all metabolites detected in the human urine were found in rats and baboons. Mice lacked MRZ 2/529 and the hydroxylated metabolite of an unidentified metabolite found in baboons and humans. The major differences in urinary memantine metabolite profile among species were the presence of MRZ 2/564 and MRZ 2/677 in rodents only, and the absence of specific isomers of MRZ 2/529 in mice or rats. MRZ 2/529 concentration was high in baboon and comprised only a minor proportion of total metabolites in human urine, and was absent in rat and mouse. The remaining differences were related to proportion of the individual metabolites in relation to total metabolic profile.

In another comparative metabolism study, the proportions of memantine, glucuronic acid conjugates and sulfuric acid conjugates in the urine were determined in mice, rabbits, dogs, baboons, and humans. The results showed similar proportions of memantine and the glucuronic acid conjugates in mouse and human urine. In the baboon, there was a greater proportion of the glucuronide conjugate compared to parent drug. Sulfate conjugates were found in the rabbit, dog, and baboon urine, only.

Memantine HCl had no effect on hepatic microsomal metabolism of aminopyrine (N-demethylase activity) *in vitro*, in microsomes prepared from rats treated with doses from approximately 12-14 mg/kg/day for 3 days. In comparison, amantadine decreased the formation of formaldehyde from aminopyrine.

A repeated dose excretion study in mice administered oral memantine (10 mg/kg) three times daily for 7 days showed nearly complete renal and fecal excretion within 48 hours of the last dose. Radioactivity recovered in expired air, sweat and tissues accounted for <1% total radioactivity dose administered. The results of additional testing showed approximately 57%-76% renal excretion and 13%-18% fecal excretion in the first 24 hours after dosing (cumulative), and 65%-88% renal and 15%-28% fecal excretion in the first 48 hours after dosing (cumulative) for total excretion of 70%-93% over 24 hours and 84%-106% over 48 hours in mice. In male Sprague Dawley rats administered ^{14}C-labeled memantine HCl by oral gavage at doses from 0.5-12 mg/kg, the radioactivity was nearly completely eliminated by 24 hours after dosing. The ratio of radioactivity recovered in urine and feces was approximately 4:1, regardless of dose and number of repeated daily doses from 1-10. Urinary excretion in pregnant rabbits was 10% at 0-6 hours, 77% at 6-24 hours, 2% at 24-48 hours, and 0.35% at 48-72 hours after dosing, for a total of 89% during the period from 0-72 hours after dosing. Cumulative renal and fecal excretion combined were 31%-50% in the first 24 hours and 77%-89% in the first 48 hours after dosing in the baboon. In the miniature pig, urinary excretion accounted for 83%-85% in the first 24 hours and 89%-94% dose in the first 48 hours after dosing, the feces fraction was 2% in the first 24 hours and 5% in the first 48 hours after dosing, and total excretion in urine and feces was 85%-86% over 24 hours and 94%-99% over 48 hours after dosing. In another comparative pharmacokinetics and metabolism study, renal and fecal excretion of ^{14}C-labeled memantine was nearly complete at 48 hours after dosing in the mouse, rabbit and dog, but only 59-76% radioactivity was excreted at 72 hours after dosing in the baboon. In comparison, 47% orally administered ^{14}C-memantine was excreted at 72 hours after administration in the human study (see Preliminary pharmacokinetic investigations with ^{14}C-memantine in healthy volunteers. Report for Merz + Co. GmbH & Co., September 1983).

The saturation of the enterohepatic circulation during long-term use was investigated in an excretion study in mice administered ^{14}C-labeled memantine by single and repeated oral doses of 10 mg/kg. Radioactivity measurements in the urine, feces, and bile after 1, 10, and 27 doses (1, 3 and 9 days at 3 doses/day) showed approximately 70% excretion by the renal and fecal routes combined. Radioactivity in bile accounted for <4% with no difference in proportion in the bile after the single dose and the 27th dose. Therefore, no saturation was found in mice, under the conditions of this study.

General Toxicology: A summary of the single dose studies conducted to evaluate acute memantine toxicity is presented in the following table:

Summary of Single Dose Toxicology Studies on Memantine

Species/Strain	Test Article Dose and Route (mg/kg)	LD50 (mg/kg)*	Treatment-related Clinical Signs	Reference
Mouse (Jcl:ICR) n=8/sex/dose/route	Memantine HCl 300,420,588,823 PO 23,28,30,33,40 IV 78,109,153,214,300,420 SC	M: 498 PO F: 437 PO M: 29.7 IV F: 32.1 IV M: 206 SC F: 138 SC	Ataxia, tremor, prone position at all PO, IV, SC doses, bradypnea at 300 mg/kg PO, all IV and SC doses, up to 5h after dosing, reduced body wt gain and food consumption at all PO and SC doses, injection site cyst/crust after IV, SC dosing	Study 88102A
Rat (Jcl:SD) n=8/sex/dose/route	Memantine HCl 214,300,360,420,588 PO 28,33,40,48 IV 214,300,360,420,588 SC	M: 370 PO F: 328 PO M: 38.3 IV F: 38.3 IV M: 439 SC F: 386 SC	Ataxia, tremor, prone position at all PO, IV, SC doses, bradypnea at 300 mg/kg PO, all IV and SC doses, up to 5h after dosing, reduced body wt gain and food consumption at all PO and SC doses, injection site cyst/crust after IV, SC dosing	Study 88102A

Rat (Sprague-Dawley) n=6/sex/dose	Memantine-HCl 60,80,100,120,150 IP	LD_{50}: 79 IP LD_{10}: 50-60 IP	Ataxia at 4 min after dosing in all groups, deep sedation, depressed breathing, convulsions, circling, clonic blepharospasm, deaths within 1h, After 2h: irritation, ataxia, tremor, Straub-tail	Merz & Co. Pilot Study
Rat (Sprague-Dawley from 2 different breeders) n=8 females/dose	Memantine HCl 45,60,80,110,150 IP	Canadian: 69.8 IP German: 60-70 IP	Clinical signs identical in Charles River Canada and Interfauna, Germany rats: ataxia, slowed breathing, lateral position, at 5 minutes after dosing, Irritation and ataxia at 2 hours after dosing	Merz & Co.
Rat (Sprague-Dawley) n=5 females/dose IV, 2 females/dose IP)	MRZ 2/325 (metabolite of memantine) 40 IV and 70,140,280 IP vs memantine at 30,36,43 IV	Not identified (>280 IP) for metabolite 36 IV and 70 IP for Memantine	Reduced motility, ataxia, dyspnea, muscular hypotonia at 280 mg/kg IP, No toxicity at 40 mg/kg IV (absorption confirmed)	10550/97
Dog (Mongrel) n=1/sex/dose	D 145 5,25,50,75 PO in capsules	24h: 60 PO 48h: 50 PO (late mortality)	NOAEL 5 mg/kg PO Ataxia, tremor, apathy, lateral position, decreased food consumption, increased water consumption, no macroscopic pathology At 75 mg/kg: tonoclonic convulsions, tremor , salivation, vomiting before death, stomach and duodenal irritation in gross examination	

*M = male; F = female; C = combined; MLD = minimum lethal dose; PO = oral; IV = intravenous; SC = subcutaneous; IP = intraperitoneal; min = minutes; h = hours; wt = weight;

Single dose toxicity was studied in mice, rats and dogs. Additionally, the acute toxicity of the memantine metabolite MRZ 2/325 was evaluated in rats. In mice and rats, memantine-induced toxicity included dose-related ataxia, tremor, prone position, and bradypnea by the oral, IV, and subcutaneous (SC) routes beginning within minutes after dosing, and lasting up to 5 hours. The necropsy examination showed SC injection site edema, hemorrhage, cyst, and crust formation in both mice and rats treated subcutaneously with memantine HCl. In the mice, deaths occurred within 30 minutes of oral drug administration at doses of 420 mg/kg and higher, within 5 minutes of IV dosing at 28-30 mg/kg and higher, and within 1 hour after SC dosing at 109-214 mg/kg and higher. The LD50 levels were higher in the males than in the females by the PO and SC routes in both mice and rats. The acute toxicity of memantine HCl by the IP route was similar to that by the PO, IV and SC routes in the previous study. The treatment-related effects after IP dosing were ataxia, sedation, depressed breathing, and at high doses (80 mg/kg IP) convulsions, circular movements and clonic blepharospasm. Deaths occurred at 60 mg/kg IP and higher doses, within 1 hour of dosing. A study comparing acute toxicity in rats from two breeders, , showed no differences in clinical signs (dose-related ataxia, sedation, slow breathing, and lateral position followed by irritation and circling) and LD50 values. Single dose toxicity in the mongrel dog included ataxia, tremor, spasms and reduced sensitivity at doses of 25 mg/kg PO and higher, and tonoclonic convulsions, apathy, lateral position, decreased food consumption and increased water consumption at doses of 50 mg/kg PO and higher.

The sponsor investigated the single dose toxicity of the memantine metabolite MRZ 2/325 in rats. IV injection of 40 mg/kg MRZ 2/325 resulted in no observable toxicity; in comparison, memantine-HCl was lethal in 20% rats administered the dose of 36 mg/kg IV. By the IP route, reduced motility, ataxia, dyspnea, and muscular hypotonia, but no deaths and no local injection site reactions were observed at 280 mg/kg (in comparison the LD50 for memantine HCl was 70 mg/kg IP). Absorption was confirmed in this study.