

The repeated dose toxicity study results are summarized in the following table:

Summary of Repeated Dose Toxicology Studies on Memantine*

Species	Memantine Dose (mg/kg/d)	Duration	Mortality	Clinical Signs	Clinical Pathology	Organ Weights, Pathology: Gross & Microscopic	NOAEL/ LOAEL (mg/kg/d) (multiple of MRHD)	Reference
Mouse (B6C3F1) 5/sex/dose	0,60,120,240 dietary	14 d	0	Dose-related ↓BWG in M&F at 120 and 240	Not done	0	60/120 (15X / 29X BSA basis)	7195/92
Mouse (B6C3F1) 10/sex/dose	0,5,20,80, 160,320 dietary	13 wk	5M & 1F at 320	80-320: Dose-related ↓BWG & BW (M&F) 160: retinal blood vessel loss (2M,2F) 320: retinal blood vessel loss (3M)	320: Males: slight ↑platelets, ↑TP, albumin, AG ratio, Beta-globulin, & ↓alpha- & alpha2-globulins; Females: ↓ urine specif gravity	160: ↑rel kidney(M), ↓rel/abs heart (F) wts, endothelial vacuolation & pyknosis corneal epithelium (3F), vacuolation of kidney tubular epithelium (1F) 320: ↓rel/abs liver wt(M&F), ↑rel brain wt (M), endothelial vacuolation/pyknosis corneal epithelium,edema of substantia propria&epithelium, thickening of Descemet's membrane (10M), kidney tubular epithelium vacuolation (2F), vacuolation/focal necrosis of kidney tubular epithelium (7M)	20/80 (5X / 19X BSA basis)	7196/92
Rat (LE & SD) 6,8,8,16 males (at 0, LD, MD, HD, respectively)	0,40,80,160 dietary	6 wk	5 LE at 160, 5 SD at 160	40-160 (both species: dose-related ↓BWG, ↓ food consumption 160 (both species): hyperactivity, jumping, aggressiveness, biting, tremors, emaciation, prolapsed penis, piloerection	40: ↓BUN (LE) 80: ↓ platelets, leukocytes, lymphocytes (both species), ↓BUN,ALT (LE), ↑A/G (LE), TP (SD), albumin (SD), ↓urine pH (both species), ↑urine specific gravity (both species) 160:hematology/clinical chemistry not evaluated, ↓urine pH (both species), ↑urine specific gravity (both species)	40: ↓abs/rel liver, spleen wt(LE), ↑rel brain wt (LE) 80: ↓abs liver wt (LE), ↓abs/rel spleen wt (LE), ↑rel brain, kidney, adrenal, testes, lung wt (LE), ↓abs/rel heart wt (LE), ↑abs/rel kidney,rel brain, liver, adrenal, testes, lung & ↓ abs spleen wt (SD) 160: organ wts & histopathology not evaluated, gross pathology in LE&SD rats found dead: ↓ adipose, enlarged adrenals, small thymus, spleen, lymph nodes, testes, prostate, seminal vesicles, dark lung	<40/40 (<19X / 19X BSA basis)	SR0004
Rat (Wistar) 25/sex/dose	0,3,15,30 gavage	2 mo	0	↓BWG in M at 30, and F at 15 and 30	0	30: ↑adrenal,testicle, brain wts (M), ↑ adrenal, ovary, brain wts (F)	3/15 (1.5X / 7X BSA basis)	3-4-170-72
Rat (SD) 20/sex/dose	M:0,40,90, 135,200 F:0,30,75,120, 180 dietary	13 wk	4M at 135, 10M at 200, 7F at 180	Dose-related ↓BWG & food consumption (M&F) all doses 135(M)/120(F): hyperexcitability, aggressivity, yellow fur, ataxia, snout scabs, corneal edema & lens lesions (M&F) 200(M)/180(F): hyperexcitability, aggressivity, yellow	40-135(M)/30-120(F): ↑ BUN, AG ratio, alkaline phosphatase, AAT, ALT, urinary ketones, blood, epithelial cells, casts, ↓ proteins, albumin, globulins, urinary pH, 200(M)/180(F): ↑ phosphorus, alkaline	90(M)/75(F): ↑adrenal & kidney wts, ↓thymus wt, alopecia 135(M)/120(F): ↑adrenal & kidney wts, ↓ thymus, testes, uterus wts, alopecia 200(M)/180(F): alopecia, lymphoid lesions (reversible), testicular & epididymal lesions (not reversible), pulmonary	<40/40(M) 30(F) (<19X-19X / 15X – 19X BSA basis)	442/003

				fur; ataxia, snout scabs, focal eye opacities corneal edema, lens lesions (1M,6F)	phosphatase, ↓ proteins, albumin, globulins, urine volume	macrophages (reversible)		
Rat (SD) 10/sex/dose	0,20,90,180 dietary	13 wk	1M at 90, 12M at 180 9F at 180	20: Corneal opacity (3M,1F) 90: ↓BWG & food consumption (M&F), Corneal opacity (1M,1F) 180: ↓BWG & food consumption (M&F), unkempt fur, emaciation, hypersensitivity, perigenitourinary smudge, staggering, punctate corneal opacity (5M,7F), focal corneal adhesion (1M), detached iris w/ hemorrhage (1M), vascularization of cornea (1F)	20: 0 90: ↑ hemoglobin, hematocrit, seg neutrophils, A/G ratio, albumin, alpha2 globulin, urine volume, ↓ WBC, lymphocytes, triglyceride, Ca, protein, urea nitrogen, creatinine, gamma globulin, alpha1 globulin, urine pH, Na, K, Cl, specific gravity 180: ↑ hemoglobin, hematocrit, seg neutrophils, GOT, GPT, LDH, RBC, urine volume, ↓ WBC, lymphocytes, reticulocytes, triglyceride, free fatty acid, glucose, total protein, urine specific gravity, pH, Na, K, Cl	20: inflammatory cell infiltration in substantia propria & Ca in cornea in 1M 90: ↑rel lung wt, atrophy of thymus, testes, seminal vesicle, prostate, lung swelling in 1M, swollen ovaries in 1F, 180: ↓ abs/rel thymus, spleen, ovary, uterus wt, ↑ abs/rel lung wt, ↓ abs/rel testes & epididymides wts in recovery M, emaciation, atrophy of thymus, spleen, mesenteric lymph nodes, ovaries, uterus in 1F, 90-180: multiple lesions (cytoplasmic vacuolation, foam cells, degeneration, necrosis, atrophy, enlargement) in many organs/tissues (particularly liver, kidneys, muscle, brain, thyroid, thymus, lungs, reproductive organs) in most animals	<20/20 (<10X / 10X BSA basis)	B-1717
Dog (Beagle) 2/sex/dose	0,2,5,10 PO in gelatin capsules	3 mo	0	10: ↓BWG, tremor, apathy after each dose escalation, opal turbidity of cornea	0	10: edema in hypophysis (1F), small Langerhans islets w/incipient fibrosis (F)	5/10 (8X / 16X BSA basis)	3-2-171-72
Baboon (wild) 3/sex/dose	0,2,4,8 PO gavage	13 wk	0	Slight ↓ BW and food consumption at all doses, no effect on BWG, dose-related quietness, nervousness, huddled posture, glazed eyes, ptosis, unsteadiness, limb tremors; at HD: vomiting on Day 1, no ophthalmologic effects	2-4: ↑ thromboplastin time 8: ↑ thromboplastin time, ↓ urine volume, pH	4: ↓ abs adrenal wt, ↑ abs thyroid wt, 8: ↓ abs adrenal wt, ↑ abs thyroid wt, ↓ abs kidney wt	0/2 (0X / 3X BSA basis)	PTX 46/8612 38
Rat (SD) 18/sex/dose	0,10,20,40 PO gavage	6 mo	0 treatment-related	Dose-related mucoid feces, corneal opacities, red eye discharge, swollen eye, ↓ BW and food consumption at 20,40, ↓ BWG at 10,20,40 (dose-related), no treatment-related ophthalmologic effects	10: ↓ creatinine 20: ↑ basophils, alkaline phosphatase, A/G ratio, phosphorus, urine epithelial cells 40: ↑ basophils, monocytes, glucose, alkaline phosphatase, A/G ratio, phosphorus, urine volume, epithelial cells ↓ eosinophils, creatinine	10: ↑ rel kidney and liver wts 40: ↑ abs/rel kidney & liver wts, ↓ abs/rel spleen, thyroid wts, liver deformity/nodule (2M,1F), mammary cyst (1F),	<10/10 (<5X / 5X BSA basis)	N00217 1A
Dog (Beagle) 6/sex/dose	0,3,9,18 PO capsule	6 mo	2 HDM	18: in the dogs that died, convulsions, incoordination, pacing, rapid respiration, hypoactivity, tonic rigidity, prostration.	18: ↑ mean percent lymphocyte count (F), mean cholesterol (M)	No treatment-related effects on organ wts, gross and microscopic observations in dogs that survived; In dogs that died: dark	9/18 (15X / 29X BSA basis)	244006

				vocalization		red GI contents, pituitary cyst		
Rat (Wistar) 15/sex/dose	0,3,15,30 PO gavage	12 mo	0 treatment- related	3: ↓ BWG & food consumption 15: ↓ BWG & food consumption 30: ↓ BWG & food consumption, ↓ water consumption (M) No treatment-related ophthalmologic effects Slight treatment- related ↓ vitality, response to environment	All hematology changes within historical control range, no treatment- related effects on clinical chemistry, urinalysis	3: ↓ abs liver wts (M&F), abs kidney wts (M&F), abs spleen wts (F), abs adrenal wts (F), spermiogenesis disturbance/vacuolar degeneration in germinal epithelium (1M) 15: ↓ abs liver wts (M&F), abs kidney, spleen, adrenal wts (M&F), spermiogenesis disturbance/vacuolar degeneration in germinal epithelium (1M) 30: ↓ abs liver kidney, spleen, adrenal wts (M&F), ovary wts (F), ↑ rel heart wt (F), moderate hemosiderin accum in macrophages in lung (1M), spermiogenesis disturbance/vacuolar degeneration in germinal epithelium (2M)	<3/3 (<1.5X / 1.5X BSA basis)	Not provided
Rat (SD) 30/sex/contr ol & HD 20/sex/LD & MD	M: 0,20,40,70 F: 0,15,30,50 dietary	52 wk	0	LD: ↓BW, BWG, food consumption, ↑ water consumption MD: ↓BW, BWG, food consumption, ↑ water consumption HD: ↓BW, BWG, food consumption, ↑ water consumption dirty fur, diffuse/focal hairloss No treatment-related ophthalmologic effects	LD: ↓ urine specific gravity (F) MD: ↑ packed cell & mean corpuscular volume (F), ↓ lymphocytes (M), ↑ urine volume (M&F), ↓ specific gravity (M) & pH (M&F) HD: ↑ packed cell & mean corpuscular vol (F), ↓ lymphocytes (M), ↑ urine volume (M&F) & ketones (M), ↓ specific gravity & pH (M&F)	LD: mottled kidneys (1M), mineralization (11M,9F), tubulointerstitial nephritis (8M) MD: small testes (1M), mottled kidneys (6M), renal papillary congestion & hemorrhage (16M,9F) & pigment accumulation (2M,1F) & mineralization (20M,14F), tubulointerstitial nephritis (11M,1F) HD: alopecia (16F), small testes (6M), mottled kidneys (9M) with dark focus/enlargement/pale kidneys (M), pale focus in lungs (M&F), renal papillary congestion & hemorrhage (20M,15F) & pigment accumulation (19M,9F) & mineralization (17M,13F), tubulointerstitial nephritis (14M,2F), ↑ pulmonary histiocytosis (M&F), cutaneous acanthosis (10F), ↑ corneal epithelial thickness, abnormal lysosomal storage in ganglion cells & retinal pigment epithelial cells After recovery: kidney papilla congestion or hemorrhage, pigment accumulation, mineralization in papilla, tubulointerstitial nephritis, histiocytosis in lung (HD M&F)	<15-20/15- 20 (<7X-10X / 7X - 10X BSA basis)	442/007

Dog (Beagle) 2/sex/dose	0.2,5,18 PO capsules	52 wk	0	18: tremors, apathy, dehydration after dose escalation, ↓BWG (M&F), ↓ food consumption (M&F) opal clouding of cornea (months 2-8)	18: ↑ alkaline phosphatase at 6(39%), 9(85%), 12 (75%) months (M&F)	2: ↑ rel adrenal wt (M&F) 5: ↑ rel thyroid wt (M&F) 18: ↑ abs/rel thyroid wt (M&F), rel adrenal, liver & kidney wt (M&F)	5/18 (8X / 29X BSA basis)	Not provided
Baboon (wild) 4/sex/dose	0,2,4,8 PO gavage	52 wk	0	2: Vomiting (1 animal) 4: Vomiting (2 animals), ptosis 8: Vomiting (9 animals), quietness, ptosis, huddled posture Vomiting within 1st days of dosing, quietness and huddled posture within first weeks of dosing, ptosis throughout dosing	All changes within historical range	4: Moderate ↑ erythroid cells in 1F without anemia or microscopic pathology 8: Moderate ↑ erythroid cells in 1M and 2F, without anemia or microscopic pathology	<2/2 (<3X / 3X BSA basis)	PTX 48/8854

*M = male; F = female; LD = low dose; MD = mid-dose; HD = high dose; LE = long Evans; SD = Sprague Dawley; d = day(s); w = week(s); mo = months; NOAEL = lowest no adverse effect level; LOAEL = lowest adverse effect level; BWG = body weight gain; BW = body weight; BSA = body surface area basis in mg/m²; TP = total protein; AG = albumin globulin; rel = relative; abs = absolute; wts = weights; BUN = blood urea; ALT = alanine transaminase; seg = segmented; WBC = white blood cell count; GOT = glutamic oxalacetic transaminase enzyme (AST); GPT = glutamic-pyruvic transaminase enzyme (ALT); LDH = lactate dehydrogenase; RBC = red blood cells;

The results of the 14-day dose-range-finding study B6C3F1 mice administered memantine HCl (dietary) at doses of 60-240 mg/kg/day for 14 days showed dose-related reduced body weight gain in the males and females at 120 and 240 mg/kg/day. Food consumption was increased in the males (dose-related) and reduced in the females (without dose-relationship) at all doses studied. There were no treatment-related deaths, clinical signs, organ weight changes and gross pathology findings. The histopathology examination of the eyes was negative for toxicity.

A palatability study was conducted in rats administered memantine HCl (3-81 mg/kg/day for 15-16 days) mixed into powdered diet. The results showed minimal effects on coordination in the rota-rod test and slightly reduced food consumption and growth rate at the highest dose. The corresponding blood levels were below those predicted in clinical treatment, except that at the high dose the blood levels were 3X-4X clinical therapeutic levels. There was a non-linear increase in serum memantine with increasing dose (factors of 28 in the males and 19 in the females as the dietary dose increased from 9 to 81 mg/kg). The plasma levels were higher in the females than in the males at all doses studied. In another palatability study, memantine HCl doses of 120 and 180 mg/kg/day in the diet resulted in dose-related interference with coordination in the rota-rod test, reduced food consumption, reduced growth rate, and weight loss. When the dose was increased to 250 mg/kg/day PO, food consumption was reduced to a level permitting a maximum memantine intake of 220 mg/kg/day PO. There were no sex differences in plasma levels at doses ranging from 120 to 250 mg/kg PO. In conclusion, the MTD by the oral route given by admixture in the diet was estimated to be approximately 200 mg/kg/day in the rat, when based on palatability.

Subchronic studies were conducted in mice, rats, dogs, and baboons. In a 13-week toxicity study in B6C3F1 mice administered memantine HCl at doses of 5-320 mg/kg/day (dietary, n=10/sex/dose), there were deaths in 1 HD female and 5 HD male mice, during the 5th through the 11th weeks of treatment. Body weight gain was reduced at 80, 160, and 320 mg/kg/day when compared to controls, and to a greater extent in the males (10%, 14%, and 21% lower than control gain, respectively, at 80, 160, and 320 mg/kg/day) than in the females (6%, 11%, and 12%, respectively, at 80, 160, and 320 mg/kg/day). Loss of retinal blood vessels was observed

in 2 males and 2 females at 160 mg/kg/day, and in 3 males at 320 mg/kg/day. There were slight, but statistically significant increases in platelets, total protein, serum albumin, albumin-globulin ratio, and beta-globulin, and decreased alpha1- and alpha2- globulin in the high dose males, and decreased urine specific gravity in high dose females. Organ weight changes were observed at the doses of 160 and 320 mg/kg/day. In the males, there were treatment-related decreases in relative liver and kidney weights, and an increase in brain weight. Relative and absolute heart and liver weights were observed in the females. The histopathology exam showed treatment-related effects in the eye and kidney. Endothelial vacuolization and pyknosis of superficial epithelial cells of the corneal epithelium were observed in 3/10 females, and vacuolization of the tubular epithelium of the kidney in 1/10 females at 160 mg/kg/day. The findings at the high dose were endothelial vacuolization, pyknosis of superficial epithelial cells of the cornea, edema of the substantia propria, superficial edema of the epithelium, focal epithelial defect, different thickness of epithelium, and thickening of Descemet's membrane in all males. Additionally, vacuolization of the tubular epithelium of the kidney was observed in 2 HD females, and focal vacuolization of the tubular epithelium in the tubulus contortus and focal necrosis of the tubular epithelium of the kidney were observed in 7 HD males. Memantine HCl intake was demonstrated at levels comparable to the intended doses. The MTD was 80 mg/kg/day (19X the MRHD of 20 mg/d in a 60 kg patient on a BSA basis) in the male and female mice, based on decreased body weight gain, loss of retinal blood vessels, organ weight changes, and eye and kidney histopathology at the doses of 160 and 320 mg/kg/day (39X-78X the MRHD).

In a comparative toxicity study, male Sprague Dawley (albino) and Long Evans (pigmented) rats were administered memantine HCl in the diet at daily doses of 40-160 mg/kg/day for 6 weeks. There were no differences in the mortality rate between the pigmented (Long Evans, LE) and albino (Sprague Dawley, SD) rats (5/16 rats of each species at the high dose of 160 mg/kg/day). Treatment-related clinical signs in the rats that died and in the high-dose rats that survived were hyperactivity, jumping, aggressiveness, biting, tremors, emaciation, prolapse of penis, piloerection, staggering, ptosis, soiled perinasal region, and self-biting, persisting for approximately 1 week to 10 days during the recovery period. There was a reversible, dose-related reduction in body weights throughout the study without species differences (final differences from controls of 12%, 18%, and 37%, respectively, in the LE rats and 9%, 15%, and 50%, respectively, in the SD rats at the doses of 40, 80, and 160 mg/kg/day). Food consumption was reduced at all doses throughout treatment without species differences, and increased from recovery Day 4 until the end of recovery. Test article intake was confirmed at slightly below the intended doses in both species. There were no abnormalities observed in the anterior segment, ocular media, and fundus at 40 and 80 mg/kg/day in either species (HD rats were not evaluated) in the ophthalmoscopy examination. The hematology evaluation showed dose-related decreased platelets, leukocytes and lymphocytes, with similar severity in both species, at the highest dose evaluated (80 mg/kg/day). There were minor species differences in treatment-related clinical chemistry effects, with decreased BUN and ALT and increased A/G ratio in the LE rats, and increased total protein and albumin in the SD rats. Both species showed dose-related decreased urinary pH and increased specific gravity at 80 and 160 mg/kg/day. Organ weight changes were more evident (low and mid-doses) in the pigmented than in the albino rats (mid-dose only). High dose rats were not evaluated for organ weights. In the pigmented rats, there were treatment-related decreases in absolute and relative liver, spleen, and heart weights and increased relative brain, kidney, adrenal, testes and lung weights. In the albino rats, there were treatment-related increases in absolute and relative kidney weights, increased relative brain, liver, adrenal, testes and lung weights, and decreased absolute spleen weights. Gross pathology abnormalities

were found in the high dose rats only, and included dry subcutaneous tissue, decreased adipose tissue, enlarged adrenals, small thymus, mesenteric lymph nodes, testes, prostate, and seminal vesicles, and dark lung at end of treatment in both species. At the end of recovery, the spleen was enlarged in the pigmented rats and small in the albino rats, the thymus was small in the albinos and prostate and seminal vesicles were small in the pigmented rats, but both species showed small testes and epididymides. No treatment-related effects were observed in the histopathology examination in either species. The doses studied were approximately 19X-78X the MRHD of 20 mg in a 60 kg patient on a mg/m² basis. A NOAEL was not determined in this study.

Memantine HCl by esophageal intubation at doses of 3-30 mg/kg/day for 8 weeks resulted in a dose-related reduction in body weight gain that was statistically significant at the high dose in male (19%) and female (11%-17%) SPF-Wistar rats. There were statistically significant increases in adrenal, testicle and brain weights in the males, and adrenal, ovary and brain weights in the females, at the high dose. The NOAEL was 3 mg/kg/day (1.5X the MRHD of 20 mg/d in a 60 kg patient on a mg/m² basis). The MTD was 15 mg/kg/day in the male rats (7X the MRHD) and <15 mg/kg/day in the female rats based on body weight gain reductions and increases in adrenal, brain and gonad weights.

Dietary administration of Memantine HCl in Sprague Dawley rats for 13 weeks at doses of 40, 90, 135, and 200 mg/kg/day in the males and 30, 75, 120, and 180 mg/kg/day in females resulted in deaths in one male given 135 mg/kg/day, 4 males given 200 mg/kg/day, and 2 females administered 180 mg/kg/day. The treatment-related clinical signs were observed at 135-200 mg/kg/day in the males and 120-180 mg/kg/day in the females, and included hyperexcitability, aggressiveness, dirty fur, ataxia, and dark scabs on the snout. There were dose-related reductions in body weights (8%, 20%, 33%, and 25% in the males, at 40, 90, 135, and 200 mg/kg/day, respectively, and 7%, 17%, 34%, and 18% in the females at 30, 75, 120, and 180 mg/kg/day, respectively), body weight gain, and food consumption at all doses from 40-200 mg/kg/day in the males and 30-180 mg/kg/day in the females. The treatment-related clinical chemistry findings were decreased proteins (7%-13%, at ≥ 40 mg/kg/day in males and ≥ 75 mg/kg/day in females), albumin (9%, 120 mg/kg/day, females), and globulins (10%-19%, ≥ 40 mg/kg/day in males and ≥ 30 mg/kg/day in females), and increased blood urea nitrogen (22%-24%, 135 mg/kg/day in males, 120 mg/kg/day in females), albumin globulin ratio, aspartate aminotransferase (102%-144% in 5/9 females at 120 mg/kg/day and 3/6 males at 135 mg/kg/day), alkaline phosphatase (33%-46%, ≥ 30 mg/kg/day in females), and alanine aminotransferase (40% in 6/10 females at 120 mg/kg/day). The urinalysis showed treatment-related increased ketones (males at ≥ 40 mg/kg/day), blood in the urine (males at 135 mg/kg/day and females at 120 mg/kg/day) epithelial cells (all groups) and casts (males at ≥ 90 mg/kg/day and females at 120 mg/kg/day), with decreased pH in the males at 135 mg/kg/day, and females at ≥ 75 mg/kg/day. After the recovery period, only increased urine volume was observed at the high dose. There was a dose related increase in adrenal and kidney weights and decreased thymus weight at ≥ 75 (females) and ≥ 90 (males) mg/kg/day, and decreased testes and uterus weights at the high doses. Alopecia was observed in the gross examination and lymphoid lesions, testicular and epididymal lesions, and pulmonary macrophages were observed at the high dose in the histopathology examination. A NOAEL was not determined in this study. The doses studied represented approximately 15X-19X (at the low dose) to 88X-97X (at the high dose) the MRHD of 20 mg/d in a 60 kg patient on a mg/m² basis. Based on a comparison of plasma levels

in the rats with those measured in clinical study #710200, the plasma levels at the doses studied were 3X-20X the plasma concentrations at human therapeutic doses. The MTD in this study was 40 mg/kg/day in the males and 30 mg/kg/day in the females, in agreement with the sponsor's conclusions.

In another 13-week study in Sprague Dawley rats, memantine administration in the diet at daily doses of 20-180 mg/kg/day resulted in deaths in 1 male at 90 mg/kg/day, and in 12/16 males and 9/16 females at 180 mg/kg/day. The treatment-related clinical signs were hypersensitivity, decreased movement and staggering, chromodacryorrhea, unkempt fur, and emaciation secondary to malocclusion-induced malnourished state. Body weights were reduced 21% and 27% in the males and females, respectively, at 90 mg/kg/day, and 64% and 27% in the males and females, respectively, at 180 mg/kg/day; body weight reduction was reversed in the recovery period. Food consumption was also reduced, reversibly, at the 90 and 180 mg/kg/day doses. Drug intake was measured at 19, 84, and 161 mg/kg/day in the males and 19, 88, and 153 mg/kg/day in the females in the 20, 90 and 180 mg/kg/day dose groups, respectively. There were treatment-related adverse effects in the cornea, that were probably related to decreased blinking induced by memantine, and included membranous/striate opacities, punctate opacities, focal adhesion or detachment of the cornea or iris, and vascularization in one or both eyes, that were only partially reversed during the recovery period. Treatment-related hematology effects were observed at the 90 and 180 mg/kg/day doses, and included increased hemoglobin, red blood cells, neutrophils, reticulocytes, and hematocrit, and decreased white blood cells and lymphocytes. At the end of the recovery period, decreased lymphocyte ratio and increased neutrophils and mean corpuscular volume were observed. Memantine administration resulted in several changes in clinical chemistry in the mid- and high-dose animals that are considered to be related to malnutrition, including decreases in triglyceride, glucose, calcium, total protein, urea nitrogen, creatinine, alpha-1 globulin fraction and gamma globulin fraction, and increased A/G ratio, albumin fraction and alpha-2 globulin fraction. However, these findings in addition to increased GOT, GPT, and LDH may be related to microscopic changes in the liver, kidneys and skeletal muscle. At the end of the recovery period, decreased LDH, triglyceride, A/G ratio, chloride, and albumin, and increased gamma globulin fraction were still evident. Treatment-related effects on the urinalysis were decreased pH, specific gravity, and excretion of sodium, potassium and chloride.

The results of the necropsy showed treatment-related increased relative and absolute lung weight, and slight decreases in absolute and relative thymus, spleen, ovary, and uterus weights. After the recovery period, decreased absolute and relative thymus, testes and epididymides weights were observed, in the high dose animals. The gross necropsy showed treatment-related atrophy of the thymus, spleen, mesenteric lymph nodes, ovaries, uterus, testes and epididymis in 1-2 animals in the high dose group. In the histopathology examination, multiple lesions were observed in many organs and tissues in several animals in the mid-dose group (90 mg/kg/day) and in most of the animals at the high dose (180 mg/kg/day). The treatment-related lesions included eosinophilic round bodies in neurocytes with increased Herring body materials in the hypothalamus. Eosinophilic material was found in the lung alveoli. Cytoplasmic vacuolation was observed in the neurocytes in the cerebrum, Purkinje cells in the cerebellum, parenchymal cells of the pituitary, spinal cord, Kupffer's cells and hepatocytes in the liver, parenchymal cells in the thyroid glands, renal tubular epithelium, adrenal cortical cells, urinary bladder epithelium, epithelial cells of the trachea, bronchial epithelium, alveolar epithelium, Leydig cells of the testes, epithelium of the epididymis, seminal vesicle, prostate, lutein cells of the ovaries, and

mucosal epithelium in the lamina propria mucosa in the uterus and vagina. Foam cells were found in the lung alveoli, tongue, lamina mucosa of the duodenum, jejunum, and ileum, lamina propria mucosa and vessel wall of the vagina and uterus, femoral muscle fibers, thymus, mesenteric lymph nodes, marginal zone of the spleen, glomeruli of the kidney, and vessel wall of the epididymis. There was degeneration, atrophy, or necrosis in the muscle fiber of the tongue, thymus, hepatocytes, mesenteric lymph nodes and white pulp of the spleen, seminiferous tubules, vessel wall of the epididymis, seminal vesicle and prostate, mammary gland, and skin cells. Additionally, treatment-related enlargement of the chief cells in the glandular stomach, adrenal enlargement, dilatation of the renal tubules, corneal lesions, swelling of the pigment epithelium, and hypocellularity in the bone marrow were observed. After the recovery period, only foam cells in the lung alveoli were observed in 4/6 of the mid-dose females. The target organs of toxicity were the cornea (probably secondary to reduced blinking), liver, kidneys, and muscle. Administration of high memantine doses (90 and 180 mg/kg/day) for 13 weeks resulted in microscopic lesions, including vacuolation, foam cells and degeneration or necrosis in nearly all organs and tissues in the male and female rats. The doses studied represented approximately 10X-88X the MRHD of 20 mg/day in a 60 kg patients on a mg/m² basis. The NOAEL in this study was 20 mg/kg/day PO (dietary, 10X the MRHD).

A non-GLP study in Beagle dogs administered memantine HCl in gelatin capsules at doses of 2, 5, and 10 mg/kg/day, once daily 5 days/week for 3 months showed tremor, apathy, and slight dehydration after dose escalations in the high dose group (from 5 to 7 mg/kg/day, and thereafter, 1 mg/kg/day increments every 4 days up to 10 mg/kg/day). Body weight gain was reduced 20% compared to controls at 2 mg/kg/day to 74% compared to controls at 10 mg/kg/day over the 12-week period. The ophthalmoscopy examination revealed opal turbidity of the cornea at the high dose at 2 months of dosing, which lasted through study termination. In the histopathology examination, slight edema in the hypophysis and small Langerhans islets with incipient fibrosis were observed in one high dose female dog. No histopathology examination of the eye was conducted. The doses studied represented up to 16X the MRHD of 20 mg in a 60 kg patient on a BSA basis. The NOAEL was between 5 and 10 mg/kg/day (8X-16X MRHD).

Administration of memantine HCl by oral gavage at doses of 2, 4, and 8 mg/kg/day, daily for 13 weeks in male and female baboons, produced treatment-related vomiting in the high-dose animals on the first day, and dose-related quietness, nervousness, huddled posture, glazed eyes, ptosis, unsteadiness, and limb tremors. Observations of quietness, piloerection and loss of appetite in the high dose groups during the recovery period suggested mild withdrawal effects. Body weights were reduced slightly (2%-13%) in a dose-related manner during the first week, and overall in all treated males (12%-23% without dose-relationship) and in the mid-dose (29%) and high-dose (19%) females throughout the study, without effect on body weight gain compared to controls. Food consumption was reduced during the first week in the treated males (6%-25%, not dose-related) and females (10%-34%, dose-related), but only slightly reduced when averaged over the 13-week dosing period in the males (4%-11%, not dose-related) and females (0.3%-5%, not dose-related). There were no deaths and no treatment-related effects in the ophthalmoscopy and clinical chemistry examinations. In the hematology evaluation, prothrombin time was increased 8%-16% (not dose-related, males and females combined) in Week 13. Urine volume was decreased 24% compared to controls in Week 13 at the high dose. The organ weight measurements showed decreased absolute adrenal weights (33% and 21% at 4 and 8 mg/kg/day, respectively), increased absolute thyroid weights (47% at 4 and 8 mg/kg/day), and decreased absolute kidney weights (16% at 8 mg/kg/day). The changes in relative organ weights were

similar in treated and control animals, and without statistically significant differences. The decreased adrenal and kidney weights and increased thyroid weights were without corresponding histopathological changes. There were no treatment-related effects on bone marrow and in the microscopic pathology examination. The toxicokinetic analysis confirmed absorption of memantine, with mean memantine plasma concentrations in Week 7 under the limit of detection in the low-dose groups, 22.4 and 14.0 mcg/l in the mid-dose males and females, respectively, and 102.4 and 49.9 mcg/l in the high-dose males and females, respectively. At 24 hours after the last dose, the mean plasma memantine levels were below the level of detection at 2 and 4 mcg/l, and 94.6 and 37.7 mcg/l in the high-dose males and females, respectively. Plasma and organ memantine concentrations were 2X higher in males than in females. There was no increase in plasma memantine with repeated dosing for 13 weeks. Memantine concentrations were high in bile and urine. Organ memantine concentrations were highest in lung, liver and eye, and lower concentrations were found in brain, spinal cord, lacrimal gland, parotid gland, and saliva, in order of decreasing concentration. After the 4-week recovery period, memantine was found only in the eye. No definitive organs of toxicity were identified in this study. The baboons tolerated memantine HCl well at doses up to 8 mg/kg/day (13X the MRHD of 20 mg in a 60 kg patient on a mg/m² basis) by the oral route for 13 weeks.

Six-month GLP toxicology studies were conducted in rats and dogs, and 1-year studies were conducted in rats, dogs (non-GLP), and baboons. The treatment-related clinical signs in Sprague-Dawley rats administered memantine HCl at doses of 10 (LD), 20 (MD), and 40 (HD) mg/kg/day by oral gavage for 6 months were increased mucoid feces, corneal opacities, red eye discharge, and swollen eye region. There were reductions in mean body weights at 20 and 40 mg/kg/day in the males (7% and 12%, respectively) and females (9% and 11%, respectively) when compared to controls. Body weight gains were reduced in a dose-related manner, by 12% and 21% in the males at 20 and 40 mg/kg/day, respectively, and by 17%, 23%, and 26% in the females at 10, 20 and 40 mg/kg/day, respectively. Food consumption increased in the males and females at 20 and 40 mg/kg/day. There were treatment-related increases in glucose (8%-19% in HD males and females on Days 22 and 183), alkaline phosphatase (15%-23% in MD and HD males on days 92 and 183), A/G ratio (8%-30% in the MD and HD males on days 183 and 211), phosphorus (8%-21% in the MD and HD males and females on days 92 and 183), and decreased creatinine (8% in the HD males on days 183 and 211). The changes in urinalysis parameters were observed in the absence of changes in serum urea, nitrogen and creatinine (in the females), were of small magnitude, and lacked consistency across dose groups, measurement times, and gender groups, and therefore are attributed to changes in hydration status in the rats. The organ weight measurements showed decreased absolute and relative (to brain weight) spleen weight (19%-35%) in the MD and HD male rats compared to the controls in the 13-week necropsy. After 6 months of memantine administration, there was an increase in absolute and relative kidney (13%-21% in HD males) and relative (to body weight) liver (10% in HD males) weights, and decreased absolute and relative (to brain weight) spleen (18%-22% in HD females) and absolute and relative (to brain weight) thyroid (29%-33% in HD females) weights. The recovery animals showed no treatment-related effects on organ weights. There were no treatment-related effects in the gross and microscopic examinations. The toxicokinetic analysis showed a dose proportional increase in plasma concentration. Slight accumulation was observed across doses and in both the males and the females with increasing duration of treatment. There were no differences between the males and females in plasma concentrations in the main study, although the C_{max} and AUC values were higher in the females than in the males in the 5-week toxicokinetic study. The NOAEL was 10 mg/kg/day in the male rats, and not established in the

female rats due to decreased body weight gain at the lowest dose tested. No definitive target organs of toxicity were identified, because the observed changes in chemical chemistry values and organ weights were of small magnitude, and were not consistent across timepoints or sexes. The doses studied (10 and 40 mg/kg/day) represented approximately 5X-19X the MRHD of 20 mg/day in a 60 kg patient on a mg/m² basis. The AUC(0-24h) values associated with 10 and 40 mg/kg/day were 1808-3782 and 12950-27344 ng.h/ml, respectively, in the toxicokinetic study.

The results of the 6-month toxicology study in Beagle dogs administered oral memantine HCl in gelatin capsules at doses of 3 (LD), 9 (MD), and 18 (HD) mg/kg/day showed one death in a HD male in Week 24, and one HD male was euthanized *in extremis* in Week 16. The clinical signs in the dogs that died or were sacrificed *in extremis* were incoordination, pacing, nervousness, rapid respiration, chomping, hypoactivity, tonic rigidity, prostration, and vocalization, with convulsions 4 hours after dosing in the dog found dead and unremitting convulsions in the dog that was euthanized. The gross pathology examination showed dark red gastrointestinal tract contents in the HD male dog that was found dead, and a pituitary cyst in the HD dog that was euthanized *in extremis*. No treatment-related clinical signs and no effects on body weights, food consumption, ophthalmoscopy, electrocardiography, clinical pathology, and in necropsy parameters were observed in the dogs that survived to the end of the study. There were no differences in toxicokinetic values between the male and female dogs. Memantine exposure increased with slightly greater than dose-proportionality. There were no differences in exposure with repeated dosing from Weeks 3 through 25, indicating no accumulation or induced metabolism. The main target organ of toxicity identified in this study was the CNS, based on the convulsions induced in 2 dogs given 18 mg/kg/day memantine HCl (29X the MRHD of 20 mg in a 60 kg patient on a mg/m² basis) after dose escalation from 9 mg/kg/day. The NOAEL was 9 mg/kg/day (15X the MRHD on a mg/m² basis).

In a 1-year toxicity study in SPF-Wistar albino rats administered memantine HCl by oral gavage at doses of 3-30 mg/kg/day, there were no treatment-related deaths, clinical signs, ophthalmological effects, and no effects on hearing, clinical chemistry and urinalysis. The Irwin's Activity Test showed slight treatment-related decreases in vitality and response to environment. Body weights were significantly and dose-dependently decreased compared to control body weights when measured during dosing weeks 26 and 52 at all doses tested, and overall during the 52-week study at 3 (16%), 15 (27%) and 30 (30%) mg/kg/day in the males, and at 3 (23%), 15 (21%), and 30 (25%) mg/kg/day in the females. Body weight gain was reduced in the males 13%-76% at 3 mg/kg/day in the first 36 weeks of the study, up to 179% at 15 mg/kg/day throughout the study, and 26%-96% at 30 mg/kg/day throughout the study. Body weight gain was reduced in the females by up to 134% at 3 mg/kg/day, 189% at 15 mg/kg/day, and 268% at 30 mg/kg/day throughout the study. Food consumption was reduced 9%-13% at all doses in the males throughout the study, and 5%-10% at 3 and 15 mg/kg/day in the females throughout the study. Water consumption was reduced in the high dose males by 14% compared to controls when averaged over 52 weeks. The changes in hematology values (slight increases in hematocrit, prothrombin time and coagulation time, and decreased mean corpuscular concentration) were within the range of historical control values, and were inconsistent across sexes or observation times, and therefore, are not considered to be toxicologically meaningful. The increase in prothrombin time was observed without a dose-relationship and in the absence of other changes in liver function. There were no treatment-related gross necropsy findings. The histopathology examination showed treatment-related nuclear polymorphism in the liver at 3 months but not in later examinations, suggesting initial metabolic loading. There was

spermiogenesis disturbance with vacuolar degeneration in the germinal epithelium in 1-2 treated males at each dose. No definitive target organs of toxicity were identified in this study. The doses studied represented approximately 1.5X-15X the MRHD of 20 mg in a 60 kg patient on a mg/m^2 basis. A NOAEL was not identified in this study.

The results of the 52-week dietary toxicity study in Sprague-Dawley rats given memantine HCl at doses of 20 (LD), 40 (MD), and 70 (HD) $\text{mg}/\text{kg}/\text{day}$ in the males and 15 (LD), 30 (MD), and 50 (HD) $\text{mg}/\text{kg}/\text{day}$ in the females showed no treatment-related deaths and clinical signs, except for dirty fur and focal hairloss. There were dose-related reductions in body weights at all doses (9%-24% in the males and 8%-23% in the females) and body weight gain (13%-34% in the males and 13%-36% in the females) compared to controls, that were partially reversed during the 6-week recovery period. Food consumption was reduced in a dose-related manner in the male rats and water consumption was increased in a dose-related manner in the LD, MD, and HD male and LD and MD female rats. Memantine HCl intake was confirmed by analysis of test article composition in the diet, dietary intake, and plasma drug level analysis. The treatment-related hematology changes were increased packed cell volume (MD and HD) and mean corpuscular volume (LD and HD) in the females, and decreased lymphocytes (MD and HD) in the males. Lymphocytes were decreased from 25%-34% at all timepoints measured, and the decrease was not reversible after 6 weeks of treatment-free recovery in the highest dose group. There was a treatment-related increase in urine volume with a corresponding decrease in specific gravity in the MD and HD males and females throughout dosing; both were reversed during the 6-week recovery period. Urine pH was decreased (8%-25%) in the MD and HD males and females, and ketones were increased in the HD males. The results of the gross and histopathology examinations indicated that the main target organs of toxicity by memantine HCl in the 1-year study in rats were the kidneys and lungs. Treatment-related alopecia, small testes, mottled kidneys with dark focus, enlargement or pale kidneys, pale focus on the lungs were observed with dose-related increases in incidence in the MD and HD males and females. Only small testes were observed after the recovery period, in 3 HD males. There were dose-related increases in the severity and incidence of renal papillary congestion and hemorrhage, pigment accumulation and mineralization in the kidney with tubulointerstitial nephritis in the MD and HD males and females, pulmonary histiocytosis at all dose levels in the males and females, intraalveolar amorphous material with macrophages and foamy or vacuolated cytoplasm at the MD and HD in both sexes, and cutaneous acanthosis in the HD females. Kidney papilla congestion, hemorrhage, pigment accumulation and mineralization, tubulointerstitial nephritis and histiocytosis persisted after the 6-week recovery period in most HD animals. The measurement of memantine blood levels confirmed adequate memantine intake by the animals, and suggested accumulation of the drug with increasing concentrations during the study. The doses studied represented approximately 10X-34X in the males and 7X-24X in the females the MRHD of 20 mg/day in a 60 kg patient on a mg/m^2 basis. A NOAEL was not identified in this study, due to reduced body weights and body weight gain, kidney mineralization, tubulointerstitial nephritis, and pulmonary histiocytosis in all treated rats at doses of ≥ 20 $\text{mg}/\text{kg}/\text{day}$ in the males and ≥ 15 $\text{mg}/\text{kg}/\text{day}$ in the females.

In addition to the standard histopathological examination, H&E stained sections from the retrosplenial and posterior cingulate cortices were examined for evidence of vacuolation and necrosis. There was no evidence of necrosis or progressive degeneration in these regions, although perinuclear vacuolation was observed in the cortical cells of 1-2 males and females in

the controls and in each treated group, and axonal vacuolation was observed in the cerebellum and brain stem in 1 HD male, that was attributed to formalin fixation artifacts.

Special histopathological examination of the eyes was conducted in the 52-week study in rats. The results showed no severe toxicity in the cornea except for increased corneal epithelial thickness without inflammatory reaction in the treated rats compared to controls. Electron microscopy of the retina was performed because memantine was found to concentrate in the eyes in several studies in rats, dogs, and monkeys, memantine is amphiphilic and accumulates in lysosomes which is associated with retinal damage by other drugs, corneal lesions and focal lens turbidities were observed in a previous 13-week toxicity study in rats. Also, some changes in the retina may not be observable by light microscopy, although no abnormalities were observed in the ophthalmoscopy examination in this study. Electron microscopy showed abnormal lysosomal storage in the ganglion cells and retinal pigment epithelial cells at end of treatment, that was partially reversed after the 6-week recovery period in the HD males and females. No structural damage of the retinal layers or retinal atrophy was found.

In a non-GLP 52-week toxicology study in Beagle dogs administered memantine HCl by oral capsule 5 days/week at doses of 2-18 mg/kg/day, there were treatment-related tremors, apathy, and reduced body weight gain after incremental dose-escalations in the high-dose (HD) group from 5 to 18 mg/kg/day. Slight dehydration was observed during the first 10 dosing months in the HD group. Reduced body weight gain was observed at the end of study in the HD dogs compared to controls (39% in males and female combined, 23% in males, and 55% in females). Absolute body weights were reduced at the high dose compared to controls at the end of the study (13% in males and females combined, 19% in males, and 5% in females). Food consumption was reduced 10% throughout the 52-week study in the HD males and females combined. Opal clouding of the cornea was found during the period from Months 2-8, decreasing thereafter in the HD dogs. No treatment-related effects were observed at the end of the study in the eye examinations. There were no treatment-related effects in the ECG, hematology, urinalysis, gross pathology and histopathology examinations. There was a slight increase (26%, males and females combined) in serum alkaline phosphatase at 3 months in the HD dogs (within normal range), and significantly increased alkaline phosphatase at 6 (39%), 9 (85%), and 12 (75%) months. Organ weight measurements showed increased absolute thyroid weights (46%-48%, left-right) at the HD, and increased relative (to body weight) adrenal weights at the LD (27%-23%, left-right) and HD (15%-10%, left-right), thyroid weights at the MD (33%-35%, left-right) and HD (63%-64%, left-right), and liver (16%) and kidney (20%-17%, left-right) weights at the HD (males and females combined). Definitive target organs of toxicity and a NOAEL were not identified in this study. The doses studied represented approximately 3X-29X the MRHD of 20 mg memantine HCl in a 60 kg patient on a mg/m² basis.

There were no deaths in male and female baboons administered memantine HCl by oral gavage at doses of 2-8 mg/kg/day, daily for 52 weeks. The treatment-related clinical signs were dose-related vomiting for the first several days of the treatment period, quietness during the first 26 weeks of dosing, huddled posture during the first several weeks of dosing, and ptosis throughout the study. There were no treatment-related effects on body weights, food consumption, ophthalmoscopy, urinalysis parameters and organ weights. However, body weights were slightly reduced in the mid-dose (MD, 4 mg/kg/day) and high-dose (HD, 8 mg/kg/day) males and low-dose (LD, 2 mg/kg/day), MD, and HD females during the 1st dosing week. There were several apparent treatment-related changes in the hematology parameters (decreased hemoglobin and

MCHC, increased MCV and neutrophils), although all values were within the range of historical control values and the changes were of very low magnitude, and therefore, are not considered to be of toxicological significance. The clinical chemistry analysis showed slightly decreased alpha2 globulin (33% at the LD, MD, and HD in Week 51), decreased beta globulin (20% at the LD, MD and HD in Week 13, and 18% at the LD and MD, and 9% at the HD in Week 51), decreased globulin (13% at the MD and HD in Week 13, and 14%-21% at the LD, MD, and HD in Week 51), decreased T4 (22% at the HD in Week 51), and decreased cortisol (27% at the HD in Week 51). The changes in thyroxine were without corresponding changes in organ weights and histopathology. The gross pathology observations showed no treatment-related effects at the terminal examination, although pale raised foci on the mucosal surface of the fundus of the stomach and dark or red mucosal discoloration in the mucosa of the fundus of the body of the stomach were observed in the high dose females after the 4-week recovery period. Examination of the bone marrow showed moderate increases in erythroid cells in 1 mid-dose female and in 1 high dose male and 2 high dose female baboons, without anemia or microscopic pathology indicating marrow stimulation, and no differences from controls after the recovery period.

The toxicokinetic measurements confirmed absorption of the test article in the baboons, and there was no evidence of accumulation. There were no differences in plasma memantine levels between the males and females. The mean bile memantine concentrations were 11 and 15 mg/l in the males and females given 8 mg/kg/day memantine HCl, respectively, at the end of the study. No memantine was found in the bile after the 4-week recovery period. Measurements of organ tissue concentrations (mcg/g) of memantine in the high dose animals showed test article in the lung (14.3 in males, 21.4 in females), eye (13.1 in males, 16.7 in females), urine (8.8 in males, 8.4 in females), liver (7.0 in males, 10.2 in females), spinal cord (5.2 in males, 5.3 in females), brain (3.6 in males, 4.7 in females), feces (2.5 in males, 2.7 in females), lachrymal gland (1.6 in males, 2.7 in females), parotid gland (1.3 in males, 2.1 in females), and prostate gland (0.7 in males). After the 4-week recovery period, memantine was detected in the eye only, at a mean concentration of 0.96 mcg/g. No definitive target organs of toxicity were identified in the 1-year toxicity study in baboons. Memantine was well tolerated by the baboons at doses of up to 8 mg/kg/day in this study. The doses studied represented approximately 3X-13X the MRHD of 20 mg in a 60 kg patient on a BSA basis.

Genetic Toxicology: The following *in vitro* and *in vivo* studies were conducted to evaluate the genotoxic and clastogenic potential of memantine:

Genotoxicity Studies with Memantine

Study	Test System	Dose Levels	Route	Study No.	Response
Reverse mutation	<i>S. typhimurium</i>	250-2000 µg/plate	<i>In vitro</i>	PTX 8/80764	Negative: reversion site AT base pair tester strain not evaluated
Reverse Mutation	<i>S. typhimurium</i>	78-5000 µg/plate	<i>In vitro</i>	89101M	Negative
Gene mutation	Chinese Hamster V79 Cells	3-300 µg/plate	<i>In vitro</i>	219510	Equivocal: Increased number of mutants at 300 µg/ml in Tests 1 (4.6X) and 2 (5.5X) w/S9. No increase in Tests 3 and 4
Chromosomal aberrations	Human lymphocytes	0.01-0.30 mg/ml	<i>In vitro</i>	219521	Negative
Metaphase analysis	Rat spermatocytes	75-300 mg/kg	PO	PTX 12/82309	Negative
Micronucleus test	Mouse bone marrow	20-80 mg/kg	IP	5525-M-06496	Negative

In the first of 2 Ames tests, memantine HCl was negative for mutagenicity in *Salmonella typhimurium* strains TA 1535, TA 1537, TA 1538, TA 98, and TA 100 at concentrations from 250-2000 mcg/plate in the presence and absence of metabolic activation with S9 under the conditions tested. However, in the first study, there was insufficient description of the methods, test article purity and stability, counting method, and incubation times, no replicates were assayed, incubation and sampling times were not provided, criteria for positive results were not described, and tester strains with the AT base pair (*E. coli* WP2, *S. typhimurium* TA 102) at the primary reversion site were not evaluated, and therefore the study is not considered to be valid. In a second Ames test in the strains *Salmonella typhimurium* TA 100, TA 1535, TA 98, and TA 1537, and *Escherichia coli* WP2uvrA, there was no bacteriostatic activity by the test article at up to 5000 mcg/plate. Therefore, 5000 mcg/plate was chosen to be the highest dose for the mutagenicity study. No reproducible, dose-related increases in the numbers of revertants (number of colonies/plate) to 2X the control numbers were observed, in the presence and absence of metabolic activation with S9 mix. The numbers of revertants were increased by more than 2X over controls in all positive controls.

In the Gene Mutation Assay in Chinese Hamster V79 cells, no statistically significant increases in the numbers of mutants were observed in the cells treated with the solvent control (Minimal Essential Medium) or solvent control with DMSO. There was a concentration-dependent increase in mutation rate in experiments I and II, at memantine HCl concentrations of 10, 30, 100, and 300 mcg/ml, in the presence of metabolic activation. In Experiment I, the number of mutant colonies per 10^6 cells at the highest concentration of 300 mcg/ml was 2.24X the number in the negative control flasks, and 4.61X the number of mutant cells in the negative control with DMSO flasks, in the presence of metabolic activation. In Experiment II, there was a significant increase in number of mutant colonies per 10^6 cells at the highest evaluable concentration (100 mcg/ml) only, of 5.5X the number of mutant colonies observed in the negative controls, and no dose relationship was observed, in the absence of metabolic activation with S9 mix. However, in the presence of metabolic activation in Experiment II, there was a significant, concentration-related increase in the number of mutant colonies per 10^6 cells, of up to 10.1X the number in the negative controls and 3.69X the number in the negative controls with DMSO, at the highest dose of 300 mcg/ml. No memantine-induced increases in mutant colonies were observed in Experiments III and IV at the concentrations of 100-300 mcg/ml in the presence of S9. Significant increases in number of mutations were observed in the positive control-treated flasks. The dosing was considered to be adequate due to excessive cytotoxicity at concentrations of 300 mcg/ml and higher. The sponsor concluded that the increase in mutation rates at the highest doses in the presence of metabolic activation were probably due to random effect (Experiment II), or that the mutation rate was within historical range of $5/10^6$ - $45/10^6$ cells (Experiment I), and therefore that memantine HCl was negative for point mutations at the HGPRT locus in V79 cells. Although memantine was negative in Experiments III and IV, conducted to prove the relevance of the results from the first 2 experiments, the mutagenic potential of memantine HCl cannot be ruled out because of the positive findings in Experiments I and II in the presence of S9 mix. The results of the Gene Mutation Assay in Chinese Hamster V79 cells are considered to be equivocal.

In the Chromosome Aberration Assay in Human Lymphocytes, there were no significant increases in chromosome breaks, fragments, deletions, exchanges, disintegrations, and gaps by memantine HCl at any concentration up to 0.1 mg/ml with and without metabolic activation with

S9 for 24 hours and without S9 for 48 hours, and at up to 0.3 mg/ml in the presence of S9 for 48 hours (aberration rates in the memantine-treated cells 0.00%-3.00% compared to 1.00%-2.50% in the negative controls). The positive control articles ethylmethanesulfonate and cyclophosphamide significantly increased the number of cells with structural chromosome aberrations (20% each), with and without metabolic activation. Therefore, in agreement with the sponsor's conclusion, memantine HCl was negative for clastogenicity at concentrations up to 0.1 (with and without S9 at 24 hours and without S9 at 48 hours) and 0.3 mg/ml (with S9 at 48 hours), under the conditions tested.

Male Sprague-Dawley rats were administered 37.5-150 mg/kg memantine HCl by oral gavage, twice, 24 hours apart (total doses 75, 150, and 300 mg/kg), in the metaphase analysis assay. There was no evidence of mutagenic potential, including chromatid gaps, breaks, translocations, and exchanges, dicentric chromosomes, acentric chromosomal fragments, minute chromosomal fragments, chromosome rings, and complete metaphase pulverisations in the rat spermatocytes in the metaphase stage of cell division. In comparison, the positive control article, mitomycin C, significantly increased the mean number of aberrant cells per 50 metaphases including and excluding gaps. In agreement with the sponsor's conclusions, memantine HCl was negative for clastogenicity in the metaphase analysis assay in rats.

Memantine HCl was negative for clastogenicity in the mouse micronucleus test at doses of 20, 40, 60, and 80 mg/kg IP; no induction of micronuclei in polychromatic erythrocytes of bone marrow in mice were observed at up to 80 mg/kg IP on 2 consecutive days. Memantine was lethal in 13/15 female and 2/15 male mice at the high dose, and there were no deaths at the lower doses. There were no statistically significant increases in the frequency of micronucleated PCE by memantine HCl at any dose (from 10-80 mg/kg IP) or timepoint (24 and 48 hours). The mean range of frequencies of micronucleated PCE in the treated groups was 0.8-1.3 at 24 hours and 1.2 at 48 hours, compared to 1.6 at 24 hours and 1.2 at 48 hours in the negative controls, and 43.4 in the positive control treated mice. Therefore, memantine tested negatively in the in vivo micronucleus test under the conditions tested in concurrence with the sponsor's conclusions.

The genotoxic potential of the major human memantine metabolite, MRZ 2/325 was evaluated in the Ames Test in 5 strains of *Salmonella typhimurium*, including 4 strains that have GC pairs at the primary reversion site and 1 strain (TA 102) that detects cross-linking mutagens. No increases in number of revertants/plate were observed at any concentration of MRZ 2/325, from 10-10000 mcg/plate compared to control frequencies, with or without metabolic activation with S9 in any *S. typhimurium* strain tested. In agreement with the sponsor's conclusion, MRZ 2/325 was negative in the Ames test, under the conditions of this study.

Carcinogenicity: Carcinogenicity studies were conducted in B6C3F1 mice and Sprague Dawley rats using dietary administration. In the mice, memantine administered for 113 weeks at up to 40 mg/kg/day in the diet (10X the MRHD of 20 mg in a 60 kg patient on a mg/m² basis, AUC data not available) produced dyspnea in the high dose females, and no effects on the end-of-study survival rate or cause of death. Body weights were 6% lower than the controls (statistically significant) in the high dose male mice at the end of the dosing period. There were no treatment-related effects on food and water consumption, eye examination, auditory function, hematology and clinical chemistry, bone marrow, organ weights and gross pathology. The standard histopathology examination showed increased adrenal nodular hyperplasia in the high dose males, rib marrow fibrosis in the high dose females, increased Harderian gland ectasia in the

high dose males, lympho-histiocytic infiltration in the kidney in the low dose and high dose males and in the lung in the high dose females, brown pigment in the submaxillary lymph node in the high dose males, and follicular hyperplasia in the urinary bladder in the mid dose males and high dose males and females.

There were no drug-related increases in neoplastic lesions in the memantine-treated mice. Re-examination of the microscopic slides by the Pathology Working Group (PWG) of the _____ conducted after inconsistencies were detected in the original final report, confirmed the original conclusion that no treatment-related increase in the incidence of neoplastic lesions indicating carcinogenic potential by memantine were present. Re-evaluation by _____ (see Amendment to Pending NDA dated September 3, 2002, Study Report 6277-146) showed no positive trends for common and rare tumors. There were significant negative trends for adrenocortical adenoma and bronchial-alveolar adenoma in the lungs in the male mice, and malignant pleomorphic lymphoma in the hematopoietic system and pituitary combined adenoma/adenocarcinoma in the female mice. Additionally, there were significant treatment-related decreases in the incidence of pituitary adenoma, combined malignant lymphocytic/pleomorphic lymphoma in the hematopoietic system in the females.

A third evaluation of the mouse microscopic slides was conducted by a consultant pathologist at _____ (Study _____-432005) to resolve the differences between the original study pathologist diagnoses and those of the Pathology Working Group with regard to the incidence of malignant lymphoma. The results of the microscopic examination by _____ concurred with those of the PWG, showing no treatment-related effects on the incidence of lymphomas and hematopoietic neoplasms in the male and female mice. In conclusion, there was no evidence of carcinogenic potential by memantine HCl at doses of up to 40 mg/kg/day for 113 weeks in B6C3F1 mice, under the conditions of this study. The high dose represented approximately 10X the MRHD of 20 mg in a 60 kg patient on a mg/m² basis (AUC data not available).

In the rats, memantine administered for 128-129 weeks at up to 40 mg/kg/day in the diet (19X the MRHD of 20 mg in a 60 kg patient on a mg/m² basis, reduced to 20 mg/kg/day [10X MRHD] after Week 71 due to marked decreases in body weights and toxicity that included kidney lesions and dyspnea, AUC data not available) produced dyspnea in the mid-dose males and high dose males and females, and no effects on the end-of-study survival rate or cause of death. Body weights were significantly lower than the controls in the high dose male and female rats throughout the dosing period. Mean body weights were reduced 16% and 19% in the male and female rats, respectively, at 40 mg/kg/day up to week 71, and 11% and 7%-22% in the males and females, respectively at 20 mg/kg/day from week 71 to the end of the study, compared to controls. There were no treatment-related effects on food and water consumption, eye examination, auditory function, hematology and clinical chemistry, bone marrow, organ weights and gross pathology. The standard histopathology examination showed increased incidence, when compared to controls, of kidney medulla mineralization in the high dose males and females, foamy macrophages in the lung in the high dose males and females, follicular hyperplasia in the cervical lymph node in the high dose females, giant cells in the testes of high dose males, and cystic glandular hyperplasia in the uterus in the high dose females. A detailed histopathology examination of the kidneys, conducted by _____ (Study 7280a/92), showed a statistically significant increase in renal medulla mineralization (mild to marked in the lumen and

epithelium of the collecting tubules, also called dystrophic mineralization), at the high dose in the male and female rats.

No significant treatment-related increases in neoplastic effects were observed in the rats, under the conditions of this study. The microscopic slides were re-examined by the 6-member Pathology Working Group (PWG)

_____ which supported the original conclusion. Re-evaluation by _____ (see Amendment to Pending NDA dated September 3, 2002, Study Report 6277-146) showed no significant positive trends in either common or rare tumors. There were significant negative trends for pancreas islet adenocarcinoma and pancreas islet adenoma with adenocarcinoma, thyroid follicular adenoma/adenocarcinoma, benign pheochromocytoma in the adrenals, thyroid c-cell adenoma, and fibroma (all organs) in the males. In the female rats, there was a significant negative trend for benign mammary fibroadenoma/adenoma and combined mammary fibroadenoma/adenoma/adenocarcinoma. A detailed histopathology examination of the kidneys, conducted by _____ (Study 7280a/92), showed no increase in the incidence of neoplastic lesions, nor differences in the type of neoplastic lesions in the kidney in memantine-treated rats when compared to the controls.

The carcinogenicity studies in mice and rats used adequate numbers of animals, and showed adequate survival, parameters evaluated and durations of treatment. The sponsor demonstrated stability and homogeneity of memantine in the animal chow and drug absorption was verified in blood samples. It does not appear that an MTD was reached in the female mice; however the doses were acceptable, based on the results of previous chronic toxicity studies and the results of the toxicology evaluations in the carcinogenicity studies.

The results of these studies were presented to the Executive CAC committee on July 22, 2003. The committee agreed that the doses in the carcinogenicity study in rats were adequate, and that the study was negative for carcinogenicity. The Committee agreed that the doses for the male mice were adequate based on toxicity in the high dose group (body weight changes in the males) and agreed that even though a MTD was not reached in the female mice, the high dose was within 1/4-1/2 of a MTD, and considering adequate doses were used in the rats and male mice, and no increases in tumors were seen in these studies, the mouse study is acceptable. The committee agreed that there were no drug-related increases in tumors.

Reproductive and Developmental Toxicology: An oral fertility study with memantine HCl in rats, embryo-fetal developmental studies in rats and rabbits, and a peri- and post-natal development study in rats were conducted by the sponsor for this NDA. In the study on fertility and early embryonic development (Segment I) in Wistar/HAN rats, memantine administration 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 mg/m² basis) resulted in significantly reduced body weights and body weight gain in the high dose F0 males and females, and decreased body weights in the high dose F1 pups, F1 dams and F1 parent males. Food consumption and testes weights were also reduced in the high dose F0 male rats. There were no treatment-related effects in the F2 pups. The NOAEL for maternal toxicity was 6 mg/kg/day PO. Memantine had no effect on male and female fertility and reproductive performance in the F0 male and female rats or their offspring (F1), and no effect on pup development in the F1 and F2 pups, except for increased incidence of non-ossified cervical vertebrae in the high dose F1 fetuses, associated with decreased fetal body weight and retarded

development. The NOAEL for adverse effects on fertility and early embryonic development in rats was 18 mg/kg/day PO.

The results of the study on embryo-fetal development (Segment II) in rats administered memantine HCl at doses of 2, 6, and 18 mg/kg/day from gestation days 6 through 15, showed a slight increase in the incidence of dumbbell shaped thoracic vertebral body at the high dose of 18 mg/kg/day (4.2% compared to 2.3% in the controls) that was statistically significant, but within the range of historical control data and without relationship to dose. There was a slight dose-related increase in the incidence of dumbbell shaped cervical vertebral body, that was not statistically significant and was within the range of historical control data. The NOAEL for maternal toxicity was 6 mg/kg/day, based on significantly reduced food consumption (reduced 25% compared to controls). Body weights were reduced 5%, body weight gains were reduced 35%, and food consumption was reduced 7%-18% in the high dose dams compared to the controls. The NOAEL for teratogenicity in rats was 18 mg/kg/day PO under the conditions of this study (9X- the MRHD of 20 mg/day in a 60 kg patient on a mg/m² basis).

The effects of memantine on embryo-fetal development (Segment II) were also studied in the rabbit (3, 10, and 30 mg/kg/day PO, 3X, 10X, and 29X the MRHD of 20 mg/day in a 60 kg patient on a mg/m² basis). The clinical signs of unsteady stance, bewildered appearance, lethargy, hunched posture, dilated pupils, pilo-erection, cold ears, and decreased fecal output at the high dose suggested decreased well-being in the maternal rabbits. Body weights and food consumption were decreased on treatment-days 6-10 and 6-13, respectively, in the high dose maternal rabbits. There were no treatment-related effects on the in-life observations, and fetal anomalies and variations. Malformations were observed in 10 fetuses in the treated groups (0.0%, 4.6%, 0.6%, and 4.1% at 0, 3, 10, and 30 mg/kg/day, respectively). The incidence of malformations had no relationship to dose, and were within the range of historical background incidence for the laboratory. The malformations included lumbar-sacral meningocele, hydrocephaly, lumbar scoliosis, dilated aortic arch with interventricular septal defect, bilateral lenticular opacity, and encephalocele at the low dose, sacral meningocele 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. The NOAEL values were 10 mg/kg/day PO for maternal toxicity and 30 mg/kg/day PO for embryo-fetal toxicity in this study. Based on the results of this study and the historical background incidence of the malformations observed, memantine HCl was negative for embryo-fetal toxicity in rabbits, under the conditions of this study.

In the peri- and post-natal (Segment III) study in rats administered memantine HCl at doses of 2, 6, and 18 mg/kg/day by oral gavage from gestation day 15 through lactation day 20 (1X, 3X, and 9X the MRHD of 20 mg/d in a 60 kg patient on a mg/m² basis), the results showed reduced mean body weights (up to 7%), body weight gain (21%), and food consumption (9.5%) in the high dose dams compared to controls, during the gestation period. Pup weights were decreased approximately 5% throughout the 20-day post partum period at the high dose. There were no treatment-related effects on number of females paired, mated, pregnant, and bearing pups, number of implantations per dam, number of dams rearing pups, number of pups per dam, breeding loss, duration of gestation, parturition and lactation behavior in the dams, and on external examination results, sex ratio, and general findings of pups during rearing, and on necropsy findings in the dams and pups. There were no treatment-related effects on pup

development (pinna unfolding, incisor eruption, onset of coat development and eye opening). The NOAEL values were 6 mg/kg/day PO for maternal toxicity and 18 mg/kg/day PO for prenatal and postnatal development in rats under the conditions of this study.

Special Toxicology Studies: Special toxicology studies were conducted to investigate potential ocular toxicity by memantine administration in rats and dogs, neurological toxicity in mice, rats, and baboons, local tolerance in Beagle dogs, and sensitization in guinea pigs.

Ocular Toxicity:

Special studies and histological examination of the eyes were performed, based on the results of previous studies that showed affinity of memantine for melanin *in vitro* and in whole body autoradiography in pigmented rats, high drug content in pigmented tissues and eyes in rats, dogs, and monkeys, and slow release of memantine from pigmented tissues. Also, memantine is amphiphilic and accumulates in lysosomes which is associated with retinal damage by other drugs, and corneal lesions and focal lens turbidities were observed in a 13-week toxicity study in rats.

The sponsor reported no effects of oral memantine HCl on lacrimation in the Shirmer's test in male and female Beagle dogs given sequentially increasing doses (4 to 20 mg/kg/day), daily for 1 month. However, the original data were not provided with the study report in the NDA submission, and therefore this conclusion cannot be supported by Agency review at this time.

In the first of three comparative studies in Sprague-Dawley (albino) and Long Evans (pigmented) rats, slit lamp examination of the eyes showed no effects in the albino rats administered memantine HCl at 180 mg/kg/day in the diet for 8-10 weeks. Lens opacities in the anterior or posterior suture region and superficial cortex with waterclefts or faint vacuolations were observed in 7/30 memantine-treated pigmented rats at the end of the treatment period. The photo-evaluation showed an increase in constant density of the cornea and a non-significant increase in lens capsular density, but no change in optical density and nuclear density, and no visible opacities in the lens and corneas during the first half of the treatment period in the albino rats. In the pigmented rats, there were treatment-related increases in the incidences of corneal density and lens capsular density compared to controls from mid-treatment to the end of the treatment period, but there was no treatment-related change in nuclear density. These results indicated significant cataract formation in the pigmented but not in the albino rats, at a dose associated with 30% mortality (180 mg/kg/day for 10 weeks, dietary) and toxicity including reduced body weight gain and food consumption, and clinical signs of lethargy and aggressiveness. The histopathology results will be reported in a separate submission. The dose studied represented 88X the MRHD of 20 mg in a 60 kg patient on a mg/m² basis. A NOAEL for ophthalmic toxicity was not determined in this study.

In a second comparative toxicity study, memantine hydrochloride by the dietary route for 6 weeks in pigmented and albino rats produced dose-related corneal lesions at the mid-dose (120 mg/kg/day) and high dose (180 mg/kg/day), that included bilateral corneal dystrophy in 1 high dose albino rat, and in 3 mid-dose and 3 high dose pigmented rats. There was only a slight increase in unilateral corneal dystrophy in the pigmented compared to albino rats. The corneal dystrophy was associated with occasional vascularization and edema due to decreased lacrymal secretion or reduced blinking, or to high concentration of memantine metabolites in tear fluid

and Harderian gland secretion. There was a slight increase in local opacities in the anterior part of the lens at the high dose in the pigmented rats. Special histological examination of the eyes 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 considered to be related 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 rats were without corresponding increases in local pathology in the eye tissues.

Although drug plasma levels were higher and there was greater lethality in the pigmented rats (2 deaths at 120 mg/kg/day and 7 deaths at 180 mg/kg/day) than in the albino rats, a similar incidence of clinical signs (hyperactivity, fur staining and piloerection), body weight reduction (15-17%, 27-29%, and 50-55% at 80, 120, and 180 mg/kg/day, respectively), reduction in food consumption (11-20%, 20-23%, and 50-58% at 80, 120, and 180 mg/kg/day, respectively), organ weight changes, gross pathology and histopathology findings were found in both strains. There were higher memantine concentrations in the pigmented skin, total eye, cornea, iris, vitreous body, retina and bulbus than in those tissues in the albino animals. 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. The species differed slightly in the kidney findings, with higher incidence of increased vacuolation of the tubular cells in the papilla with foamy configuration in the albino rats.

The results of an additional 6-week dietary administration study at doses of 0, 120 and 160 mg/kg/day (58X and 78X the MRHD of 20 mg in a 60 kg patient on a mg/m² basis) in Sprague Dawley (albino) and Long Evans (pigmented) rats, showed no treatment-related effects in the eye, optic nerve and Harderian gland in either species. There were few toxicologically relevant differences between the albino and pigmented strains in mortality, clinical signs, reduction in body weight gain and food consumption, test article intake, eyeball opacities, hematology and clinical chemistry parameters, organ weights, and gross and microscopic abnormalities at the end of the study. The high dose albino and pigmented rats showed similar histopathology effects, including swollen Kupffer cells in the liver, vacuolar degeneration and dilatation of the renal tubules, degeneration and necrosis of muscle fibers, vacuolation of nerve cells in the cerebrum, pons, and cerebellum (Purkinje cells). Atrophy of the tubular epithelium and necrosis of the collecting tubules in the kidneys were observed in the HD albino rats, and atrophy of hepatocytes was observed in the HD pigmented rats. No species-related differences in pharmacokinetic parameters were found.

Neuronal Toxicity:

The sponsor conducted investigations in mice, rats, and baboons to determine the potential of memantine to produce neuronal lesions as described by Olney *et al* (Science 244: 1360-1362, 1989) in rodents administered N-methyl-D-aspartate (NMDA) receptor antagonists. The Olney

et al studies found dose-dependent neuronal vacuolation in the multipolar and pyramidal cells in cortical layers III and IV of the posterior cingulate and retrosplenial neocortices of rats administered PCP, MK-801, ketamine and tiletamine. The 3-15 μ m vacuoles observed by Olney and his colleagues, resulting from dissolution of mitochondria and other cytoplasmic components, were evident under light microscopy as early as 2 hours after a single dose, increased in severity for 12 hours and then resolved by 18-24 hours. Two days after drug administration, neuronal necrosis could be observed in the retrosplenial and cingulate cortices in animals administered NMDA receptor antagonist doses that were higher than those associated with vacuolation (Fix *et al.*, Experimental Neurology 123: 204-215, 1993).

The results of the supplementary neurological histopathology examination in mice administered memantine HCl at doses of 5, 20, 80, 160, and 320 mg/kg/day in the diet for 13 weeks showed minimal to moderate neuronal vacuolation in the brain stem and cerebellum in all of 5 male mice administered the highest dose of 320 mg/kg/day (78X the MRHD of 20 mg in a 60 kg patient on a body surface area basis). There were no treatment-related findings in the high dose females, nor in the mice in the lower dose groups (5-160 mg/kg/day), except for minimal focal vacuolation in the brain stem and cerebellum in 2 male mice at 80 mg/kg/day (19X the MRHD). Minimal vacuolation was observed in the retrosplenial and cingulate cortices of the control and treated mice with similar incidence and severity of lesions. However, a positive control group (e.g., MK-801-treated group) was not used in this study; therefore, it cannot be concluded from the results of this study alone that memantine HCl is negative for neuronal vacuolation characteristic of that described by Olney *et al.*, in mice.

Memantine HCl, administered by single intraperitoneal injection in female Sprague Dawley rats, induced dose-related increases in the severity and incidence of neuronal vacuolation in layers III and IV in the large multipolar and pyramidal cell cytoplasm in the retrosplenial and cingulate cortices at doses of 25 and 50 mg/kg, when examined by light microscopy at 6 hours after dosing. At 48 hours after dosing, light microscopy showed a dose-related increase in neuronal degeneration in these brain regions, associated 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. Electron microscopy showed vacuolation with dilated mitochondria and rough endoplasmic reticulum (with degranulation and vesiculation), splitting of the nuclear membrane, dilated Golgi-complex, and intracytoplasmic vacuolation in the cytoplasm of the neuronal processes with a dose-related increase in severity at the doses of 25 and 50 mg/kg IP memantine HCl at the 6-hour timepoint. 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. In comparison, light microscopy of 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 electron microscopy, large perinuclear and cytoplasmic vacuolation were observed 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 were observed at 48 hours. 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 NOAEL for IP memantine-induced neuronal vacuolation and necrosis in the retrosplenial and cingulate cortices was 12.5 mg/kg in this study.

Minimal to slight intracytoplasmic vacuolation was observed in layers III and IV of the retrosplenial and cingulate cortices of male and female rats at 6 hours after a single dose of 100 mg/kg (49X the MRHD of 20 mg in a 60 kg patient on a mg/m² basis) by oral gavage. The NOAEL was 50 mg/kg PO (24X the MRHD). When administered as a single oral dose in the diet at 100 mg/kg, memantine HCl induced minimal intracytoplasmic vacuolation in the neurons of layers III and IV in the retrosplenial and cingulate cortices, observable 6 hours after dosing in sections stained with toluidine blue, but not in the sections stained with H&E (NOAEL 50 mg/kg dietary, 24X the MRHD). No vacuolation was found in the retrosplenial and cingulate cortices in the male and female rats after repeated oral dosing by gavage and in the diet for 14 days; however minimal (1-2/section) red neurons (necrotic neurons with condensed or pyknotic nuclei, and eosinophilic plasma) were seen in 2/4 high dose males dosed by gavage (50 mg/kg/day, 24X), 3/4 high dose males dosed by dietary intake (100 mg/kg/day, 49X), 2/4 females at 25 mg/kg/day (gavage, 12X the MRHD), 4/4 females at 50 mg/kg/day (gavage, 24X), and in 3/4 females at 100 mg/kg/day (49X) dietary memantine HCl. The NOAEL 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 NOAEL 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. The female rats were more sensitive to the neurotoxic effect of memantine than were the males after acute and repeated oral intubation, showing a higher frequency of vacuolated neurons at the high dose levels (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) that was not seen in the males. No differences were observed between the males and females in the frequency and sensitivity to dose when administered by the dietary route. In comparison, single doses of MK-801 at 5 mg/kg IP induced slight to marked vacuolation in layers III and IV in the retrosplenial and cingulate cortices of both male and female rats.

There was a dose-related increase in severity of neuronal toxicity in the retrosplenial and cingulate cortices of female Sprague Dawley rats administered memantine HCl by continuous intravenous infusion. Intracytoplasmic vacuolation was observed 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. At 72 hours after an 18-hour infusion, neuronal necrosis was observed at the doses of 3.14 (1.67% neurons lesioned, mild to moderate severity) and 6.28 (5.82% neurons lesioned, moderate to marked severity). The distribution pattern of necrosis observed at 72 hours was similar to that found 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. A NOAEL for memantine-induced vacuolation and necrosis in the retrosplenial and cingulate cortices in rats was not identified in this study.

There was no histological evidence of neuronal vacuolation and necrosis in Layers III and IV of the cingulate cortices of baboons administered memantine HCl by oral gavage at the dose of 8 mg/kg/day (13X the MRHD of 20 mg in a 60 kg patient on a body surface area basis, 1X-2X the MRHD on an AUC basis), daily for 14 days. However, the study is considered to be inadequate because it lacked negative and positive control (e.g., MK-801) groups, an maximum tolerated dose (MTD) based on toxicity was not evaluated, and the retrosplenial cortices were not

examined for neuronal lesions. Therefore, it cannot be concluded with certainty that memantine HCl is negative for induction of "Olney"-type lesions in baboons. The mean plasma AUC_{0-inf} values were 2020 ng.h/ml and 3890 ng.h/ml on dosing Days 1 and 12, respectively, at the dose of 8 mg/kg/day PO in the baboons.

Local Tolerance:

Local tolerance was investigated in purebred Beagle dogs administered memantine HCl (10 mg) by single intravenous (vena cephalica antebrachii), intraarterial (arteria femoralis), intramuscular (hind limb), and paravenous (vena saphena parva) injections. The injection sites were examined at 2, 24 and 48 hours, and 14 days after dosing. The gross examinations showed slight injection site inflammation in 3/4 dogs at 24 and 48 hours after intravenous injection (vs 1/4 controls), and slight edema of the injection site in 3/4 dogs at 24 and 48 hours after paravenous administration (vs 0/4 controls). Histopathology examination revealed slight inflammation in 3/4 dogs at 24 and 48 hours after intraarterial administration (vs 2/4 in controls) and in 4/6 dogs at all timepoints examined (up to 14 days) after intramuscular administration (vs 4/6 in controls). In conclusion, single injections of memantine HCl induced a slight increase in inflammation by the intravenous route when compared to saline control, and increased edema by the paravenous route. Both effects were resolved by the time of the 14-day examination in dogs.

Sensitization:

In a skin sensitization test in female guinea pigs, three 6-hour epicutaneous induction applications, at concentrations of 0.88%, 1.75%, and 3.5% memantine HCl (volume 0.5 ml), were made to depilated flank skin, 1 week apart. A challenge application was applied two weeks after the last induction application and skin reactions were evaluated according to the method of Buehler. The results showed no sensitization reaction in any of the guinea pigs administered epicutaneous memantine HCl. In comparison, 2% 1,4-phenylene diamine dihydrochloride solution induced erythema after the challenge application in all positive control guinea pigs, indicating a positive sensitizing reaction.

Impurities:

The drug substance impurities with specifications greater than the threshold for qualification are

_____ These impurities were present in Batch R7206, used in the 2-year mouse and rat carcinogenicity studies, and in the Mouse Micronucleus test. _____ 1 was present in this batch at _____%, for a total daily intake at the high dose of 0.008 mg/kg/day in the carcinogenicity studies (safety margin for the _____% level = 0.65X-1.3X the daily human intake of 0.06 mg/day in a 60 kg patient taking 20 mg/day memantine HCl on a mg/m^2 basis). _____ was present in this batch at _____%, for a total daily intake of 0.068 mg/kg/day (safety margin for the _____% level = 5.5X-11X the daily human intake of 0.06 mg/day in a 60 kg patient taking 20 mg/day memantine HCl on a mg/m^2 basis) at the high doses. The isomers of memantine were present in this batch at _____%, for a total daily intake of 0.012 mg/kg/day in the carcinogenicity studies (safety margin for the _____% level = 1X-2X the daily human intake in a 60 kg patient taking 20 mg/day memantine HCl on a mg/m^2 basis) at the high doses. No data was available to determine if these impurities were present in the batch (R7979)

used in the reproductive toxicology studies. However, no teratogenic potential by the impurities is predicted when based on comparison of the impurities to test compounds with similar molecular structures for which reproductive toxicity data is available (Computational Toxicology Report on Memantine Impurities using MCASE-ES computational toxicology estimations). Also, the potential for reproductive toxicity in the intended patient population with Alzheimer's disease is of somewhat less concern because these patients are generally post-menopausal. For additional information on the qualification batch used in the toxicity studies, please refer to the Chemistry Review for this submission. Based on the extensive exposure of the animals to these impurities in the carcinogenicity and genotoxicity studies, the results of the toxicity studies and the safety margins represented for exposure to the impurities, these impurities are considered to be adequately qualified.

Conclusions:

Memantine activity related to the proposed indication of treatment of moderate to severe dementia of the Alzheimer's type has been demonstrated in animals. In experimental paradigms of neuroprotection and memory enhancement, memantine shortened the time to return to consciousness in animal models of head injury, decreased the number of trials in a retention session in anoxia-treated mice, improved learning in rats with internal capsule lesions, and antagonized the disturbed EEG patterns in pons ischemic 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. Decreased extent of neuronal loss in the CA1 subfield, reduced number of MAP2 positive somata indicating cytoskeletal alterations in several hippocampal areas, reduced immunoreactivity for astroglial and microglial macrophage markers GFAP and ED1, and reduced apoptotic profiles in the hippocampus, were observed in memantine-treated rats injected with beta-amyloid 1-40 (A β 1-40) to induce hippocampal neuronal degeneration, a model of degeneration observed in Alzheimer's disease patients. The mechanism of action appears to be voltage-dependent antagonism of the NMDA receptor. Memantine and MRZ 2/169, a metabolite detected in dog, but not mouse, rat, baboon, and human urine, are moderate to potent NMDA antagonists, and the metabolites MRZ 2/325 (dimethyl-gludantan), MRZ 2/371, MRZ 2/373, MRZ 2/374, and MRZ 2/375 very weakly antagonize the NMDA receptor.

Memantine HCl at high doses (24X the MRHD of 20 mg in a 60 kg patient on a mg/m² basis) produced notable CNS effects in rodents, indicated by decreased indices of awareness, mood, and motor activity, CNS excitation, and interference with posture, muscle tone, reflexes, and autonomic function in the safety pharmacology studies. Memantine increased spontaneous motility with lower potency than d-amphetamine, and increases sleeping time. Memantine decreased or abolished electroshock-induced convulsions, but increased the number of pentetrazol-induced convulsions in mice. Memantine antagonized reserpine-induced reduction in body temperature.

Memantine showed only minor cardiovascular effects in the nonclinical studies. Memantine reduced the HERG inward tail current amplitude by 13% at the highest concentrations tested (100 μ M). The cardiovascular effects of intraduodenal memantine HCl in dogs included dose-related (at 10-30 mg/kg) decreases in cardiac minute output, stroke volume, and systolic left

ventricular blood pressure. In the evaluation of renal effects, memantine HCl induced diuresis and saluresis at 40 mg/kg PO during the period from 2-5 hours after dosing in rats.

Memantine gastrointestinal effects included inhibition of intestinal motility in a dose-related manner in rats, with an ED₅₀ of 20 mg/kg PO. Memantine HCl when applied alone had a slight spasmogenic effect in isolated guinea-pig ileum, but showed concentration-related spasmolytic effects by antagonizing acetylcholine, histamine, barium chloride, and 5-hydroxy-tryptamine-induced contractions.

The potential for abuse of memantine appears to be low, based on the results of several preclinical studies on self-administration, reduction of the severity of morphine withdrawal, PCP substitution, conditioned place preference, and conditioned motor activity.

Memantine HCl was rapidly and extensively absorbed by the oral route, producing peak plasma levels within ½ - 2 hours in animals. Oral bioavailability is approximately 80% in rats, regardless of dose. There were greater than linear increases in peak plasma levels (C_{max}) and exposure (AUC) with increasing memantine dose in animals. The C_{max} and AUC values were higher in female than in male rats. The half-life was approximately 3-6.5 hours and clearance was rapid. A large volume of distribution, observed in most species suggested extensive tissue distribution. A dose-related increase in half-life and decrease in clearance was also observed in the animal studies. Distribution in rodents was primarily to the liver, kidneys, lungs, gastrointestinal tract, adrenals, testicles, and intestinal fat, with lower concentrations found in brain, muscle, lacrimal gland, Harderian gland, salivary glands, spleen and blood. The tissue concentrations were increased 2X-2.5X after repeated dosing when compared to tissue levels after administration of single doses. Memantine was distributed to the eyes, and particularly to the cornea, uvea (vascular layer of the eyeball), sclera/choroid/retina, lens and vitreous body in pigmented rats, and was detectable at trace levels 28 days after dosing. Memantine also crossed the blood brain barrier and placenta, and bound to melanin.

Memantine is metabolized by hydroxylation, N-oxidation and conjugation. The major metabolites found in human urine are MRZ 2/325 (1-N-(3,5-dimethyl)-gludantan, 19%, also identified in rat urine), MRZ 2/374 (4/8-hydroxy-regio-isomers, 18%, also found in the urine of mice, rats and baboons), MRZ 2/524 (13%, found in baboon urine), and MRZ 2/525 (total N-hydroxy-memantine, 3% in urine, also found in mice). MRZ 2/373 (3-hydroxymethyl-metabolite) was produced in mice, rats, baboons, and in low concentrations in humans. MRZ 2/375 was recovered in rat and baboon urine only, MRZ 2/564 and MRZ 2/677 was recovered only in mice, and MRZ 2/529 and MRZ 2/371 were recovered in baboon urine, only. The major differences in the 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 concentrations were high in baboons and comprised only a minor proportion of total metabolites in human urine, and was absent in rats and mice. The remaining differences were related to proportion of the individual metabolites in relation to total metabolic profile. There were similar proportions of memantine and the glucuronic acid conjugates in mouse and human urine, while in baboons, there was a greater proportion of the glucuronide conjugate compared to parent drug. Sulfate conjugates were found in rabbit, dog, and baboon urine, only. Memantine HCl had no effect on hepatic microsomal metabolism of aminopyrine (N-demethylase activity) *in vitro*.

Oral memantine was nearly completely excreted as parent drug and conjugated metabolites in the urine (65-94%) and feces (5%-28%) within 48 hours after dosing in mice, rats, rabbits, dogs, miniature pigs, and baboons. In comparison, 47% orally administered ^{14}C -memantine was excreted at 72 hours after administration in a human study (see Preliminary pharmacokinetic investigations with ^{14}C -memantine in healthy volunteers. Report for Merz + Co. GmbH & Co., September 1983). Measurement of the radioactivity in the bile of mice administered ^{14}C -labeled memantine by single and repeated oral doses accounted for less than 4% total dose. There was no difference in proportion in the bile after the single dose and the 27th dose, and therefore, no saturation of the enterohepatic circulation after repeated dosing. A comparative pharmacokinetics study showed considerably lower elimination half-lives in dogs and baboons (4.4-10.2 hours) than in humans (52-58 hours) after oral administration of memantine.

The single dose toxicity of memantine HCl is considered low in mice, rats and dogs following subcutaneous, intravenous, intraperitoneal, and oral administration. The oral median lethal doses were approximately 1X-3X higher than the subcutaneous LD_{50} values, 4X-5X higher than the intraperitoneal LD_{50} values, and 10X-14X higher than the intravenous LD_{50} values in rodents. The oral, subcutaneous, intraperitoneal and intravenous LD_{50} values were 440-500 mg/kg, 140-440 mg/kg, 70 mg/kg, and 30-38 mg/kg, respectively, in rats and mice. In dogs, the oral LD_{50} was 50-60 mg/kg. The treatment-related clinical signs at high, oral doses in animals included ataxia, tremor, prone position, bradypnea, sedation, dyspnea, muscular hypotonia, apathy, and at extremely high doses (60-150 mg/kg IP in rats and 75 mg/kg PO in dogs), convulsions preceding death.

Subchronic (2-13 weeks) oral dosing in mice, rats, dogs, and baboons was lethal at 320 mg/kg/day (78X the MRHD of 20 mg in a 60 kg patient on a BSA basis) in mice and 135 mg/kg/day (66X the MRHD) in rats. In one study, there was one death in a male rat at 90 mg/kg/day PO. The treatment-related clinical signs in the rats that survived in the subchronic studies were similar to those observed in the rat that died at doses of ≥ 120 mg/kg/day PO. The dogs showed apathy and tremors after dose escalations from 5 to 10 mg/kg/day PO, and the baboons displayed quietness, nervousness, huddled posture, glazed eyes, ptosis, unsteadiness, and tremors at 2-8 mg/kg/day PO, with vomiting on the first dosing day. Body weights, body weight gain, and food consumption were reversibly reduced in a dose-related manner in all species tested, by oral memantine HCl given for 2-13 weeks.

The main target organs of toxicity in the subchronic oral dosing studies were the CNS, kidney, and eyes. Renal toxicity was manifest by vacuolation of the kidney tubular epithelium (≥ 160 mg/kg/day) and focal necrosis of the kidney tubular epithelium (≥ 320 mg/kg/day) in mice. Also, increased blood potassium (≥ 120 mg/kg/day), reduced total protein (≥ 120 mg/kg/day) and urine pH, and increased urine specific gravity (≥ 80 mg/kg/day) were observed in the clinical pathology evaluation in rats, and decreased urine pH and increased specific gravity were observed in baboons (8 mg/kg/day). Ocular toxicity included retinal blood vessel loss, endothelial vacuolation and pyknosis of the corneal epithelium at ≥ 160 mg/kg/day, edema of the substantia propria and epithelium with thickening of Descemet's membrane at ≥ 320 mg/kg/day in mice, and corneal edema and lens lesions in Sprague-Dawley rats at ≥ 120 mg/kg/day. Increased incidences of focal eye opacities, focal corneal adhesion, detached iris with hemorrhage, and vascularization of the cornea were observed in Sprague Dawley rats at the dose of 180

mg/kg/day. In the dogs, opal turbidity of the cornea was observed at 10 mg/kg/day. There were no ophthalmoscopic effects in baboons at up to 8 mg/kg/day memantine HCl.

The target organs of toxicity in the chronic animal studies were the eyes, kidneys, and testes. Chronic oral memantine HCl administration (6-12 months) produced dose-related mucoid feces, corneal opacities, reduced body weight gain and/or reduced food consumption in rats administered doses of 3-70 mg/kg/day, reduced body weight gain and food consumption, reversible opal clouding of the cornea, and during dose escalations tremors and apathy in dogs at 18 mg/kg/day, and vomiting with ptosis and quietness at higher doses in baboons at 2-8 mg/kg/day. Elevated alkaline phosphatase levels were observed in the rats and dogs, and necropsy findings included mottled kidneys, renal papillary congestion and hemorrhage, pigment accumulation and mineralization in the kidneys, tubulointerstitial nephritis, spermiogenesis disturbance with vacuolar degeneration in the germinal epithelium, and increased corneal epithelial thickness, abnormal lysosomal storage in ganglion cells and retinal pigment epithelial cells in the rats. There was no increase in memantine-related toxicity with chronic dosing, when compared to the toxicity observed in the subchronic studies.

Memantine HCl was negative for mutagenicity *in vitro*, in *S. typhimurium* or *E. coli* in the reverse mutation assay. Memantine HCl was negative for clastogenicity *in vitro* in the Chromosome Aberration Assay in human lymphocytes at up to 0.1 mg/ml with and without metabolic activation with S9 for 24 hours and without S9 for 48 hours, and at up to 0.3 mg/ml in the presence of S9 for 48 hours. In the *in vivo* assays for clastogenicity, memantine HCl was negative in the metaphase analysis assay in rat spermatocytes at 75-300 mg/kg, and in the mouse micronucleus test at doses of 20, 40, 60, and 80 mg/kg IP.

The results of the Gene Mutation Assay in Chinese Hamster V79 cells are considered to be equivocal. There was a concentration-dependent increase in mutation rate in experiments I and II, at memantine HCl concentrations of 10, 30, 100, and 300 mcg/ml, in the presence of metabolic activation, but no memantine-induced increases in mutant colonies were observed in Experiments III and IV at the concentrations of 100-300 mcg/ml in the presence of S9. The sponsor concluded that the increase in mutation rates at the highest doses in the presence of metabolic activation were probably due to random effect (Experiment II), or that the mutation rate was within historical range of $5/10^6$ - $45/10^6$ cells (Experiment I), and therefore that memantine HCl was negative for point mutations at the HGPRT locus in V79 cells. Although memantine was negative in Experiments III and IV, conducted to prove the relevance of the results from the first 2 experiments, the mutagenic potential of memantine HCl cannot be ruled out because of the positive findings in Experiments I and II in the presence of S9 mix.

The major human memantine metabolite, MRZ 2/325 was negative in the Ames test in 5 strains of *Salmonella typhimurium*, including 4 strains that have GC pairs at the primary reversion site and 1 strain (TA 102) that detects cross-linking mutagens, at concentrations from 10-10,000 mcg/plate, in the presence and absence of metabolic activation with S9.

Memantine HCl was negative for carcinogenicity in mice treated for 113 weeks at up to 40 mg/kg/day in the diet (10X the MRHD of 20 mg in a 60 kg patient on a mg/m² basis, AUC data were not available) and rats treated for 128-129 weeks at up to 40 mg/kg/day in the diet (reduced to 20 mg/kg/day from Weeks 71 to the end of the study due to severe reduction in body weight and toxicity including dyspnea and kidney lesions, 10X the MRHD). The results of the original

evaluations were confirmed by re-examination of the microscopic slides by the Histopathology Peer Review and Pathology Working Group (PWG) (statistical evaluation of rat survival and neoplastic lesions), and (evaluation of the lymphoma histopathologic diagnosis in the mouse). The Executive CAC committee (July 22, 2003) agreed that the carcinogenicity studies in mice and rats were acceptable and showed no drug-related increases in tumors.

Memantine HCl, at oral doses up to 9X the MRHD of 20 mg/d in a 60 kg patient on a mg/m² basis, produced no adverse effects on male and female fertility and reproductive performance in male and female rats or their offspring (F1). The highest dose was associated with reduced body weights and body weight gain in the F0 males and females, and decreased body weights and increased incidence of non-ossified cervical vertebrae in the F1 pups, F1 dams and F1 parent males. The NOAEL for adverse effects on fertility and early embryonic development in rats was 18 mg/kg/day PO. In New Zealand White rabbits, there were no treatment-related fetal anomalies, variations and malformations at doses up to those associated with maternal toxicity, including lethargy, unsteadiness, and reduced body weights and food consumption. Memantine was also not teratogenic in rats. The NOAEL for teratogenicity in the rats was 18 mg/kg/day PO (9X the MRHD on a mg/m² basis) and in the rabbits was 30 mg/kg/day PO (29X the MRHD). In the study on perinatal or postnatal development in rats, decreased pup weights were observed at the dose of 18 mg/kg/day (NOAEL 6 mg/kg/day, 3X the MRHD).

The results of 3 comparative ocular toxicity studies in Long Evans (pigmented) and Sprague-Dawley (albino) rats showed a higher incidence of treatment-related lens opacities, corneal density, and lens capsular density, indicating significant cataract formation in the pigmented rats but not in the albino rats at a dose associated with 30% mortality and severe toxicity (180 mg/kg/day for 10 weeks, dietary, 88X the MRHD on a mg/m² basis). Additionally, corneal lesions were observed at a higher incidence in the pigmented rats. However, hypertrophy with foamy aspect of pigment epithelium cells in the iris, retina, and corneal epithelial cells, and erosion, keratitis, and thinning of the epithelium in the corneal stroma were observed with similar incidence in both strains, and were therefore attributed to increased concentration of memantine in the tear fluid. Memantine concentrations were higher in the pigmented rat skin, total eye, cornea, iris, vitreous body, retina and bulbus.

Memantine HCl by the IP, IV, and oral routes in rats was positive for induction of dose-related neuronal lesions in the multipolar and pyramidal cells in cortical layers III and IV of the posterior cingulate and retrosplenial neocortices, like those described by Olney *et al* (Science 244: 1360-1362, 1989) in rodents administered N-methyl-D-aspartate (NMDA) receptor antagonists. Dose-related increases in the incidence and severity of neuronal vacuolation ("Olney Lesion") in these regions were observed at 6 hours after dosing at 25-50 mg/kg IP, 7.82-15.65 mg/kg/h IV (continuous infusion), 100 mg/kg PO (single dose by gavage and dietary) memantine. Dose-related neuronal degeneration and necrosis were observed in the retrosplenial and cingulate cortices at the doses of 25-50 mg/kg IP, 3.14-6.28 mg/kg IV (18 hour continuous infusion), 25-50 mg/kg PO (gavage, repeated dose, 14 days), and 100 mg/kg PO (dietary, 14 days) in the 48-hour or 72-hour examinations. The degeneration was associated with condensed nuclei, eosinophilic plasma and dark neurons, and shrunken, necrotic neurons with eosinophilic cytoplasm and pyknotic triangular nuclei. When examined by electron microscopy, the retrosplenial and cingulate neurons in rats treated by the IP route with doses of 25 and 50 mg/kg showed vacuolation with dilated mitochondria and rough endoplasmic reticulum (with

degranulation and vesiculation), splitting of the nuclear membrane, dilated Golgi-complex, and intracytoplasmic vacuolation in the cytoplasm of the neuronal processes with a dose-related increase in severity at the 6-hour timepoint. At 48 hours after dosing, electron microscopy 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.

Female rats appear to be more sensitive to the neurotoxic effect of memantine than male rats after acute and repeated oral intubation, showing a higher frequency of vacuolated neurons in the cingulate and retrosplenial cortices at the high dose levels (100 mg/kg by single dose and 50 mg/kg/day by repeated dose), and vacuolation at the mid-dose (25 mg/kg/day repeated dose) that was not observed in the males. No differences were observed between the males and females in the frequency and sensitivity to memantine-induced neuronal vacuolation by the dietary route. The NOAEL values for induction of the neuronal vacuolation by memantine HCl in rats were 12.5 mg/kg IP (single dose), <7.82 mg/kg/h IV for 6 hours, 50 mg/kg PO (gavage, 24X the MRHD, mg/m² basis), and 100 mg/kg PO (dietary, 49X the MRHD, mg/m² basis). The NOAEL for neuronal necrosis in the 14-day repeated dose experiment was 25 mg/kg/day PO (gavage, 12X MRHD on a mg/m² basis) in males, 12.5 mg/kg/day PO (gavage, 6X the MRHD, mg/m² basis) in the females, and 50 mg/kg/day PO (dietary, 24X the MRHD, mg/m² basis) in males and females. The LOAEL for vacuolation by the IV route (7.82 mg/kg/day) corresponded to a mean plasma memantine concentration of 2508-2633 ng/ml (approximately 31X-33X the steady state plasma level of 80 ng/ml at the therapeutic dose of 20 mg/day in humans).

No treatment-related increase in vacuolation in the retrosplenial and cingulate cortices were found in mice administered memantine HCl at up to 320 mg/kg/day memantine in the diet for 13 weeks (78X the MRHD, mg/m² basis), although there was minimal to moderate neuronal vacuolation in the brain stem and cerebellum. There was no histological evidence of neuronal vacuolation and necrosis in Layers III and IV of the cingulate cortices of baboons administered memantine HCl by oral gavage at the dose of 8 mg/kg/day for 14 days (13X the MRHD of 20 mg in a 50 kg patient on a mg/m² basis, AUC_{0-inf} = 2020 ng.h/ml on Day 1 and 3890 ng.h/ml on Day 12). The neurotoxicity evaluations in the mice and baboons did not use positive control groups (e.g., MK-801-treated animals) to establish the validity of the assays. Also, the study in baboons lacked a negative control group, failed to reach the maximum tolerated dose based on toxicity (the single dose evaluated was 1X-2X the MRHD on an AUC basis) and included no evaluation of the retrosplenial cortex. Therefore it cannot be concluded with certainty that memantine is negative for induction of "Olney"-type lesions in mice and baboons, from the results of these studies alone.

Single intravenous and intraarterial memantine injections (10 mg) induced slight increases in the incidence and severity of local inflammation and paravenous administration resulted in increased edema at 24 and 48 hours after injection, that were resolved by the time of the 14-day examination in Beagle dogs. No sensitization reaction was observed in guinea pigs at the concentrations of 0.88%-3.5% memantine HCl given by epicutaneous injections.

The drug substance impurities with specifications greater than the threshold for qualification are
These
impurities were present in Batch R7206, used in the 2-year mouse and rat carcinogenicity

studies, and in the Mouse Micronucleus test. No data were available to establish that these impurities were present in the memantine HCl batch used in the reproductive toxicology studies; however, 5 model compounds with similar molecular structures were found negative for teratogenicity in mammals. Based on the extensive exposure of the animals to these impurities in the carcinogenicity and genotoxicity studies (see discussion under Overall Summary and Evaluation, above), and the safety margins represented for exposure to the impurities, these impurities are considered to be adequately qualified.

General Toxicology Issues:

The main target organs of toxicity in the animal toxicology studies were the CNS, eyes, and kidneys. The treatment-related CNS effects in rodents, decreased indices of awareness, mood, and motor activity, CNS excitation, and interference with posture, muscle tone, reflexes and autonomic function at high doses, and hyperactivity, staggering, aggressiveness, tremors, ptosis, huddled posture, piloerection, and hypothermia at extremely high doses in several species, suggest a potential for interference with cognitive, autonomic and motor functions in clinical use. The clinical safety data included reports of minor dose-related CNS effects including dizziness and confusion.

Memantine HCl induced vacuolation and necrosis in the cingulate and retrosplenial cortices in rats, similar to the NMDA receptor antagonist-induced lesions described by Olney *et al.* Although no memantine-related vacuolation and necrosis were observed in the cingulate cortex in a neurotoxicity study in baboons, the study is considered to be inadequate for several reasons. Only 4 animals were evaluated, positive and negative controls were not used, the retrosplenial cortex was not examined, and the single dose evaluated (8 mg/kg, 1X-2X the MRHD on an AUC basis) failed to show adequate toxicity indicating that it was the maximum tolerated dose. The risk of the NMDA-receptor antagonist-induced "Olney Lesion" in humans is not known, and the potential for central neuronal toxicity in humans by memantine HCl cannot be ruled out. Patients given this drug should be adequately informed of the potential for neuronal damage suggested by the results of the studies in rodents.

Ocular toxicity by memantine at high doses in the animal studies included retinal blood vessel loss, endothelial vacuolation and pyknosis of the corneal epithelium, edema of the substantia propria and epithelium with thickening of Descemet's membrane, corneal edema and increased thickness, lens lesions, abnormal lysosomal storage in ganglion cells and retinal pigment epithelial cells, and focal eye opacities in the rodents, and opal turbidity of the cornea in the dogs. These results suggest a potential for adverse ophthalmic effects in humans administered chronic, high dose memantine HCl. However, ocular assessments by the sponsor in >400 patients treated for 28 weeks with memantine HCl found no ocular abnormalities.

Renal effects in the animal studies included vacuolation and focal necrosis of the kidney tubular epithelium, suppurative nephritis, mottled kidneys, renal papillary congestion and hemorrhage, pigment accumulation, tubulointerstitial nephritis, and kidney medulla mineralization. The safety factors for renal toxicity, based on the no-effect levels and the MRHD of 20 mg/day in a 60 kg patient on a mg/m² basis, were 39X, 15X, 16X, and 6.5X in mice, rats, dogs, and baboons, respectively, in the subchronic studies, and 10X, 29X, and 13X in rats, dogs, and baboons, respectively, in the chronic studies.

Other adverse effects of memantine HCl at high doses may include constipation, indicated by dose-related decreased intestinal motility in rats, and decreased blood pressure indicated by decreased systolic left ventricular blood pressure in dogs. Potential effects of memantine in the testes were suggested by lymphocytic infiltration, testicular atrophy, spermiogenesis disturbance with vacuolar degeneration in the germinal epithelium, and giant cells in the testes in the subchronic and chronic studies in rodents at doses representing 1.5X-10X the MRHD. Potential hematopathology was indicated by increased thromboplastin time at 3X-13X the MRHD, and increased erythroid cells without anemia at 6.5X-13X the MRHD in baboons treated for 13 weeks and 52 weeks, respectively.

Memantine showed no evidence of genotoxic potential in the Ames test, the Chromosome Aberration Assay in human lymphocytes, the metaphase analysis assay in rat spermatocytes, and in the Mouse Micronucleus test. However, because the results were equivocal in the Gene Mutation Assay in Chinese Hamster V79 cells, the mutagenic potential of memantine HCl cannot be ruled out. Memantine HCl was negative for carcinogenicity in standard 2-year dietary assays in mice and rats, at up to 10X the MRHD of 20 mg/day in a 60 kg patient on a BSA basis. Memantine HCl had no adverse effects on male and female fertility in rats, no teratogenic effects in rats and rabbits, and no adverse effects on perinatal and postnatal development in rats at doses up to 18 mg/kg/day (9X the MRHD on a BSA basis), except for decreased pup weights and an increase in incidence of non-ossified cervical vertebrae in rats at the highest dose.

Three impurities were described with specifications greater than the threshold for qualification. These impurities,

are considered to be adequately qualified based on inclusion in the drug batch used in the 2-year carcinogenicity studies in mice and rats and in the Mouse Micronucleus test at levels representing adequate multiples of the human exposure. No teratogenic potential by the impurities was predicted when based on comparison of the impurities to test compounds with similar molecular structures for which reproductive toxicity data is available (Computational Toxicology Report on Memantine Impurities using MCASE-ES computational toxicology estimations).

Recommendations:

1. This NDA is considered to be approvable from a non-clinical perspective.
2. No deficiencies in the nonclinical program were identified.
3. The results of the studies on neurotoxicity in animals, showing memantine-induced vacuolation and necrosis in the retrosplenial and cingulate cortices ("Olney Lesion"), should be included in the label as described under C. Recommendations on Labeling, in the Executive Summary, above.

X. APPENDIX/ATTACHMENTS:

Addendum to review: None

Other relevant materials: None

Any compliance issues: None

**APPEARS THIS WAY
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this page is the manifestation of the electronic signature.**

/s/

Kathy Haberny
10/9/03 04:17:46 PM
PHARMACOLOGIST

Barry Rosloff
10/9/03 04:42:25 PM
PHARMACOLOGIST
I concur with the conclusions and recommendations in this
review.

Executive CAC

Date of Meeting: July 22, 2003

Committee: David Jacobson-Kram, Ph.D., HFD-024, Chair
Joseph Contrera, Ph.D., HFD-901, Member
Abby Jacobs, Ph.D., HFD-540, Member
C. Joseph Sun, Ph.D., HFD-570, Alternate Member
Barry Rosloff, Ph.D., Team Leader
Kathleen Haberny, Ph.D., Presenting Reviewer

Author of Draft: Kathleen Haberny, Ph.D.

The following information reflects a brief summary of the Committee discussion and its recommendations. Detailed study information can be found in the individual review.

NDA # 21-487

Drug Name: Memantine HCl

Sponsor: Forest Laboratories, Inc.

Background:

Memantine is an uncompetitive N-methyl-D-aspartate (NMDA) receptor antagonist with moderate affinity, that blocks pathological NMDA receptor overactivation, but not normal physiological activation, due to 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 decreasing excessive glutamate transmission 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.

Orally administered memantine HCl is rapidly and completely absorbed, and shows extensive tissue distribution in animals, crosses the blood brain barrier and placenta, and binds to melanin. Metabolism is by hydroxylation, N-oxidation and conjugation, and the metabolic profiles are similar in mice, rats, baboons, and humans. Memantine is nearly completely excreted as parent drug and conjugated metabolites in the urine, within 48 hours after administration in rodents, rabbits, dogs, pigs and baboons.

The acute oral toxicity of memantine was low in animals, with LD₅₀ values of approximately 500 mg/kg in rodents and 50 mg/kg in dogs, and included ataxia, tremor, bradypnea, dyspnea, muscular hypotonia, and convulsions at very high doses. In the safety pharmacology studies, memantine at high doses had notable CNS effects, including CNS excitation, decreased indices of awareness and motor activity, and interference with posture, muscle tone, reflexes and autonomic function. Subchronic and chronic high dose memantine was associated with CNS effects, reduced body weight gain and food consumption, and toxicity in the kidney, liver, and eyes. The no-effect doses for these effects were high, indicating a large safety margin for

clinical use. Memantine was not mutagenic in the Ames test, human lymphocytes, rats spermatocytes and in the mouse micronucleus test; the results were equivocal in Chinese Hamster V79 cells.

Mouse Carcinogenicity Study: The sponsor submitted the protocols for the mouse and rat carcinogenicity studies for review in 1992. Comments were conveyed to the sponsor by Dr. Robert Osterberg, Acting Assistant Director for Pharmacology/Toxicology, ODE, CDER (Letter dated August 27, 1992). The doses were selected based on the results of a 13-week preliminary study on oral toxicity in B6C3F1 Cr1 mice (n=10/sex/dose), at doses of 5, 20, 80, 160, and 320 mg/kg/day in the diet. The results showed reduced body weight gain at 80, 160, and 320 mg/kg/day when compared to controls, that was more pronounced in the males (10%, 14% and 21% lower than control gain, respectively, at 80, 160, and 320 mg/kg/day) than in the females (6%, 11%, 12%, respectively). Decreased food consumption (16.3%-29.3%), decreased relative and absolute liver, spleen and heart weights, increased relative kidney weights, changes in corneal epithelium, and vacuolation of the tubular epithelium in the kidney were observed at the dose of 160 mg/kg/day. The dose of 320 mg/kg/day was lethal for the male mice.

In the carcinogenicity study, memantine administered for 113 weeks at up to 40 mg/kg/day in the diet produced dyspnea in the high dose female mice, and no effects on the end-of-study survival rate or cause of death. Body weights were significantly lower than the controls in the high dose male mice throughout the dosing period. There were no treatment-related effects on food and water consumption, eye examination, auditory function, hematology and clinical chemistry, bone marrow, organ weights and gross pathology. The standard histopathology examination showed increased adrenal nodular hyperplasia in the high dose males, rib marrow fibrosis in the high dose females, increased Harderian gland ectasia in the high dose males, lympho-histiocytic infiltration in the kidney in the low dose and high dose males and in the lung in the high dose females, brown pigment in the submaxillary lymph node in the high dose males, and follicular hyperplasia in the urinary bladder in the mid dose males and high dose males and females. In the mice that died prematurely and that were sacrificed before the end of the study, lympho-histiocytic infiltration was increased in all memantine-treated males and females, suppurative nephritis and pyelitis were observed in the high-dose females, and liver hepatocyte vacuolation and necrosis were observed at high incidence in the high-dose males and females.

There were no drug-related increases in neoplastic lesions in the memantine-treated mice. Re-examination of the microscopic slides by the Pathology Working Group (PWG) _____ conducted after inconsistencies were detected in the original final report, confirmed the original conclusion that no treatment-related increase in the incidence of neoplastic lesions indicating carcinogenic potential by memantine were present. Re-evaluation by _____ (see Amendment to Pending NDA dated September 3, 2002, Study Report 6277-146) showed no positive trends for common and rare tumors. There were significant negative trends for several neoplastic lesions.

A third evaluation was conducted by a consultant pathologist _____ (Study _____-432005) to resolve the differences between the original study pathologist diagnoses and those of the Pathology Working Group with regard to the incidence of malignant lymphoma. The results of the microscopic examination by _____ concurred with

those of the PWG, showing no treatment-related effects on the incidence of lymphomas and hematopoietic neoplasms in the male and female mice. In conclusion, there was no evidence of carcinogenic potential by memantine HCl at doses of up to 40 mg/kg/day for 113 weeks in B6C3F1 mice, under the conditions of this study. The high dose represented approximately 8X the MRHD of 20 mg in a 50 kg patient on a BSA basis (AUC data not available).

The MTD does not appear to have been reached in the female mice in the carcinogenicity study. Body weight gain in the 13-week range-finding study was decreased 6% in the females at 80 mg/kg/day and 11% at 160 mg/kg/day. However, memantine HCl at the high dose of 40 mg/kg/day in the carcinogenicity study had no effect on body weights in the females. Other effects of memantine observed in the carcinogenicity study included dyspnea (10%-16% incidence) and increased incidence of nephritis and liver necrosis in the high dose female mice. In a safety pharmacology study in mice (Irwin test), memantine HCl at doses of 30 mg/kg and higher produced adverse CNS effects including decreased awareness from 30-150 minutes after dosing, indicated by decreased ability to place self after being put in different positions and decreased righting reflex. Prior concurrence of the protocol for the carcinogenicity study was implied by letter conveyed to the Sponsor after Agency review (Letter dated August 27, 1992). The high dose used in the carcinogenicity study was within approximately 1/4-1/2 the MTD in the 13-week study, and represented 8X the MRHD on a BSA basis. Memantine produced no carcinogenic signal in the rats and mice of either sex, and produced significant negative trends for several neoplastic lesions in both species. Based on these factors, the high dose of 40 mg/kg/day in the carcinogenicity study is considered adequate.

Rat Carcinogenicity Study: The sponsor submitted the protocols for the mouse and rat carcinogenicity studies for review in 1992. Comments were conveyed to the sponsor by Dr. Robert Osterberg, Acting Assistant Director for Pharmacology/Toxicology, ODE, CDER (Letter dated August 27, 1992). The doses were selected based on the results of 13-week and 6-month toxicology studies in Sprague-Dawley rats, administered memantine HCl in the diet. In the 13-week dose range-finding study, body weights were reduced by 8% in the male rats at 40 mg/kg/day, and 7% in the female rats at 35 mg/kg/day. The results of the 6-month toxicity study showed treatment-related reduction in mean body weights of 12% in the male rats and 11% in the female rats, respectively at the dose of 40 mg/kg/day. Body weight gain was reduced by 12% and 21% at 20 and 40 mg/kg/day, respectively, in the males, and by 17%, 23%, and 26% at 10, 20, and 40 mg/kg/day, respectively, in the females, in the 6-month study.

In the carcinogenicity study, memantine administered for 128-129 weeks in the diet at up to 40 mg/kg/day (8X the MRHD of 20 mg in a 50 kg patient on a BSA basis, reduced to 20 mg/kg/day [4X MRHD] after Week 71 due to marked decreases in body weights and toxicity that included kidney lesions and dyspnea, AUC data not available) produced dyspnea in the mid-dose males and high dose males and females, and no effects on the end-of-study survival rate or cause of death. Body weights were significantly lower than the controls in the high dose male and female rats throughout the dosing period. Mean body weights were reduced 16% and 19% in the male and female rats, respectively, at 40 mg/kg/day up to week 71, and 11% and 7%-22% in the males and females, respectively at 20 mg/kg/day from week 71 to the end of the study, compared to controls. There were no treatment-related effects on food and water consumption, eye examination, auditory function, hematology and clinical chemistry, bone marrow, organ weights

and gross pathology. The standard histopathology examination showed increased incidence, when compared to controls, of kidney medulla mineralization in the high dose males and females, foamy macrophages in the lung in the high dose males and females, follicular hyperplasia in the cervical lymph node in the high dose females, giant cells in the testes of high dose males, and cystic glandular hyperplasia in the uterus in the high dose females. A detailed histopathology examination of the kidneys, conducted by — (Study 7280a/92), showed a statistically significant increase in renal medulla mineralization (mild to marked in the lumen and epithelium of the collecting tubules, also called dystrophic mineralization), at the high dose in the male and female rats.

No significant treatment-related increases in neoplastic effects were observed in the rats, under the conditions of this study. The microscopic slides were re-examined by the 6-member Pathology Working Group (PWG)

— which supported the original conclusion. Re-evaluation by — (see Amendment to Pending NDA dated September 3, 2002, Study Report 6277-146) showed no significant positive trends in either common or rare tumors. There were significant negative trends for several neoplastic lesions. A detailed histopathology examination of the kidneys, conducted by — (Study 7280a/92), showed no increase in the incidence of neoplastic lesions, nor differences in type of neoplastic lesions in the kidney in memantine-treated rats when compared to the controls.

Comments: The carcinogenicity studies in mice and rats used adequate numbers of animals, and showed adequate survival, parameters evaluated and durations of treatment. The sponsor demonstrated stability and homogeneity of memantine in the animal chow and drug absorption was verified in blood samples. The doses were acceptable, based on the results of previous chronic toxicity studies and the results of the toxicology evaluations in the carcinogenicity studies.

Executive CAC Recommendations and Conclusions:

Rat:

- * The Committee agreed that the doses were adequate.
- * The Committee agreed that study was negative for carcinogenicity.

Mouse:

*The Committee agreed that the doses for the male mice were adequate based on toxicity in the HD group (BW change in males) and agreed that even though an MTD was not reached in the female mice, the high dose was within 1/4-1/2 of an MTD, and considering adequate doses were used in the rats and male mice, and no increases in tumors were seen in these studies, the mouse study is acceptable.

- *The Committee agreed that there were no drug-related increases in tumors.

David Jacobson-Kram, Ph.D.
Chair, Executive CAC

cc:\

/Division File, HFD 120

/Barry Rosloff, Ph.D., Team leader, HFD-120

/Kathleen Haberny, Ph.D., Reviewer, HFD-120

/Melina Griffis, CSO/PM, HFD-120

/ASeifried, HFD-024

**This is a representation of an electronic record that was signed electronically and
this page is the manifestation of the electronic signature.**

/s/

David Jacobson-Kram
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