- Histopathology: None

Results:

- Clinical signs: Vomition was noted in the treated animals. Decreased spontaneous activity, paralytic gait, tremor, salivation, and/or soft stools/diarrhea were reported for these animals with increasing frequency correlated to increased dose.
- Body weights: No differences from controls were reported.
- Food consumption: No differences from controls were reported.
- Hematology: The 300 mg/kg males showed increased leukocyte counts with neutrophilia. The 300 mg/kg females showed a slight decrease in the lymphocyte ratio.
- Clinical chemistry: A slight increase in ALT was reported in high dose male
- Gross pathology: No treatment related lesions were reported.

Key Study Findings: The sponsor concluded that these doses were appropriate for the 4 week oral toxicity study.

Study Title: <u>15 Day Oral (Gavage) Preliminary Toxicity Study of HMR 3647 in the Cynomolgus Monkey</u>
Study No: 1453-552-036
Vol #, and page #: 31, page 224
Conducting laboratory and location: [redacted]
Date of study initiation: 4/17/97
GLP compliance: As per the German Chemical Law and OECD guidelines
QA- Report: No
Methods:
Dosing:

- species/strain: Cynomolgus monkey
- #/sex/group or time point: 1
- age: 3-8 years of age
- weight: ~3 kg
- dosage groups in administered units: 200 or 300 mg/kg/d at initiation; 100 mg/kg/d from Day 28-41
- route, form, volume, and infusion rate: Orally by gavage at 4 mL/kg/d.

Drug, lot: Batch 17
Formulation/vehicle: 0.5% methylcellulose
Observations and times:

- Clinical signs: Daily
- Body weights: Weekly
- Food consumption: Daily
- EKG: Once pre-dose and Weeks 2 and 6 for rate, PR, QRS and QT intervals, blood pressure
- Hematology: Pre-dosing and Days 7, 16, 21, 33 and 41 with GLDH, AST, ALT and GGT on Days 19, 21 and 26 additionally.
- Clinical chemistry: Same as for hematology
- Gross pathology: All animals at termination
- Organs weighed: Adrenals, brain, heart, kidneys, liver, ovaries, pituitary, spleen, stomach, testes, thyroids
- Histopathology: Evaluated tissues: cecum, colon, duodenum, epididymides, esophagus, gall bladder, heart, ileum, jejunum, kidneys, liver, lungs, mesenteric lymph nodes, pancreas, rectum, spleen, stomach and thymus.
- Toxicokinetics: Samples were taken on Days 1, 4, 30, 33, 35, and 38 at 24 hours post-dosing and on Days 28 and 41 at 0, 1, 2, 4, 6, and 24 hours post-dosing.

Results:

- Clinical signs: The high dose male died on Day 8 with marked dehydration following diarrhea. No histopathologic evidence of systemic toxicity was found. The female 300 mg/kg had emesis, diarrhea, emaciation and apathy. At 200 mg/kg/d, emesis and diarrhea were reported.

As a result of the clinical signs, animals were not dosed from Day 8 to Day 27 and were given 100 mg/kg/d for Days 28-41. Signs at this dose were emesis, hypothermia and salivation.

- Body weights: Weight loss was reported at the 200 and 300 mg/kg/d doses (8 or 20%, respectively). No weight effects were found at 100 mg/kg/d.
- Food consumption: Feed consumption was markedly decreased (~50%) at the 200 and 300 mg/kg levels. Intake was moderately decreased (up to 41%) at 100 mg/kg/d.
- Cardiovascular evaluations: No drug-related differences from controls were reported.
- Hematology: No treatment-related differences from controls were noted.
- Clinical chemistry: AST and ALT were elevated in all animals in a dose-related fashion. GLDH was also increased. Levels returned towards normal in the non-dosing period. Total bilirubin was increased in the 200 mg/kg female and the 300 mg/kg male.

After the drug-free period, GLDH and ALT were increased again in the 100 mg/kg/d animals.

- Organ weights: The premature decedent (300 mg/kg male) had relative kidney and liver weights that were markedly increased (~50%).
- Gross pathology: No treatment-related gross lesions were reported.

- Histopathology: "Possibly treatment-related moderate tubular atrophy in the kidney of 2/3 surviving animals." The sponsor attributed the finding to severe dehydration and poor physical condition during the first week of dosing. As it appears that the poor condition was a direct effect of dosing, the histologic finding is considered treatment related. It is reasonable to consider the kidney to be a potential target organ in the cynomolgus monkey.
- Toxicokinetics: A large increase in C_{24h} was found, especially at 200 mg/kg/d. The time course during the second dosing period demonstrated Cmax at 2-6 hours post-dosing. An increase in Cmax and AUC was found between Days 1 and 14 (a factor of 2). A slight increase in C_{24h} was noted between Days 1 and 14.

Key Study Findings: The sponsor concluded that HMR 3647 was not well tolerated at 200 or 300 mg/kg/d, but it was moderately well tolerated at 100 mg/kg/d in the cynomolgus monkey.

Study Title: <u>28 Day Oral (Gavage) Administration Subchronic Toxicity Study of HMR 3647 in the Cynomolgus Monkey</u>
Study No: 1485-552-037
Vol #, and page #: 32, page 1
Conducting laboratory and location: [redacted]
Date of study initiation: 1/13/98
GLP compliance: Yes for the [redacted] portion of the study
QA- Report: Yes
Methods:
Dosing:
- species/strain: Cynomolgus monkeys
- #/sex/group or time point: 5
- age: 4-8 years of age
- weight: Males: 3.2- 5.4 kg; females: 2.5- 3.5 kg
- dosage groups in administered units: 0, 30, 60 or 120 mg/kg/d
- route, form, volume, and infusion rate: Gavage at 4 mL/kg/d

Drug, lot: Batch 17
Formulation/vehicle: 0.5% methylcellulose
Observations and times:
- Clinical signs: Daily
- Body weights: Weekly
- Food consumption: Estimated daily and evaluated weekly
- Ophthalmoscopy: Pre-dosing and at study termination including electroretinography pre-dose and Week 4.
- EKG: Pre-dosing and Week 4 of dosing approximately 1 hour post-dosing.
- Hematology: Pre-dosing, Day 7 and Week 4
- Clinical chemistry: Pre-dosing and Week 4
- Urinalysis: Pre-dosing and Week 4
- Gross pathology: All animals at study termination

- Organs weighed: Adrenals, brain, heart, kidneys, liver, lungs, ovaries, pituitary, spleen, testes, thymus, thyroid, prostate, uterus, epididymides
- Histopathology: Tissues listed below from all animals on study. Liver and kidney were collected for electron microscopy.
- Toxicokinetics: Days 1 and 28 of dosing at 0 and 30 minutes, 1, 2, 4, 8 and 24 hours post-dosing. Reported as 98/10208/CN.

Results:

- Clinical signs: Emesis, and soft feces were noted for the high dose animals and poor physical condition for 2 high dose animals towards the end of the dosing period. These animals also had elevated ALT and AST levels and electrolyte imbalances for the last week on study. Occasional emesis and soft feces were noted in all treatment groups, but "not as extensive as in Group 4 animals and therefore are not considered to be related to treatment with the test article."
- Body weights: High dose males showed significant weight loss (~10% during the first week) during the study. High dose females showed an initial weight loss.
- Food consumption: Consumption was decreased in the high dose animals when compared to controls.
- Ophthalmoscopy: No significant treatment-related lesions were found.
- Electrocardiography: No significant treatment-related differences from controls were reported.
- Hematology: No significant treatment-related findings were reported.
- Clinical chemistry: Significantly increased ALT (~3x on Day 7, ~4x on Day 28) and AST (~1.5x on Day 7, ~3x on Day 28) levels were reported in the high dose animals. There was also a significant increase in total bilirubin for males in the 60 (1.8x) and 120 (2.3x) mg/kg/d groups. Creatinine levels were increased (1.4x) in the high dose males on Day 7. The sponsor did not consider the last 2 parameter increases to be treatment-related as the Day 28 values were comparable to controls.
- Urinalysis: No significant treatment-related differences from controls were appreciated.
- Organ Weights: Relative liver weights were statistically and toxicologically significantly increased in the high dose animals (~120% of control values). Absolute liver weights of high dose females were also significantly increased (122%).
- Gross pathology: No treatment-related gross lesions were reported.
- Histopathology: No systemic organ toxicity was revealed on histologic evaluation.
- Toxicokinetics:

Mean Values		Males			Females		
Parameter	Day	30 mg/kg/d	60 mg/kg/d	120 mg/kg/d	30 mg/kg/d	60 mg/kg/d	120 mg/kg/d
Cmax (mg/L)	1	0.383	1.82	5.17	0.89	1.91	5.51
	28	0.68	3.18	7.62	1.48	2.04	8.4
Tmax (hr)	1	1-4	2-8	4-8	1-4	2-8	4-8
	28	0.5-4	4	2-8	1-2	4	2-8
AUC $_{0\text{-}24hr}$ (mg/L.hr)	1	2.06	14.6	66	3.4	11.4	47.9
	28	4.0	23.0	99.3	6.0	12.1	97
C_{24} (mg/L)	1	LOQ	LOQ	0.148	LOQ	LOQ	0.117
	28	LOQ	LOQ	0.69	LOQ	LOQ	0.32

The between animals variability was moderate in this study. Cmax and AUC were higher for females at the low dose only. Cmax and AUC did not increase in proportion with dose with the ratios always higher than the ratio of the doses.

The Cmax and AUC increased between Days 1 and 28 by a factor of ~2.

Key Study Findings: The sponsor considered HMR 3647 to be well tolerated at 30 and 60 mg/kg/d and at 120 mg/kg/d to be moderately well tolerated for 28 days in the cynomolgus monkey. They determined the NOEL for this oral study to be 60 mg/kg/d.

Histopathology Inventory for IND #

Adrenals	X
Aorta	X

Bone Marrow smear	X
Brain	X
Cecum	X
Cervix	X
Colon	X
Duodenum	X
Epididymis	X
Esophagus	X
Eye	X
Gall bladder	X
Gross lesions	X
Heart	X
Ileum	X
Injection site	X
Jejunum	X
Kidneys	x
Liver	X
Lungs	X
Lymph nodes, submandibular	X
Lymph nodes, mesenteric	X
Mammary Gland	X
Optic nerves	X
Ovaries	X
Pancreas	X
Parathyroid	X
Pituitary	X
Prostate	X
Rectum	X
Salivary gland	X
Sciatic nerve	X
Seminal vesicles	X
Skeletal muscle	X
Skin	X
Spinal cord	X
Spleen	X
Sternum	X
Stomach	X
Testes	X
Thymus	X
Thyroid	X
Tongue	X
Trachea	X
Urinary bladder	X
Uterus	X
Vagina	X

Study Title: **Plasma Concentrations of RU 76363 and RU 71094, Imidazolo-Pyridine, After Repeated Oral Administration of 60 mg/kg/L of HMR 3647 for 28 Days to Five Male Cynomolgus Monkeys**
Study No: 98/10762/CN
Vol #, and page #: 33, page 203
Conducting laboratory and location: HMR, Cedex, France
Date of study initiation: In vivo study #1485-552-037 (28 Day Oral Study in Monkey) reviewed above.

- Toxicokinetics: The 60 mg/kg/d males were bled at 0, 0.5, 1, 2, 4, 8 and 24 hours post-dosing on Days 1 and 28 and 24 hours after dosing on Days 8, 15 and 22. The purpose of the study was to determine the changes in plasma concentrations of the metabolites in monkeys.

Results:

- Toxicokinetics: No marked day to day variation was noted for the two metabolites. A slight increase in the RU 76363 AUCs was noted over time and exceeded the AUCs for the parent compound while the imidazol-pyridine metabolite composed ~24%.

Since the metabolites' molecular weights are significantly different, the sponsor expressed the AUCs as μmol/L/hr. When evaluated this way, the AUCs are essentially the same with 1 mole of each being formed at 40.4 and 38.5 μmol/L/hr for RU 76363 and RU 71094, respectively.

Study Title: Ultrastructural Study of the Liver in the 1 Month and 3 Month Oral Toxicity Studies of HMR 3647 in Rats and Dogs
Study No: 99/10977/TX
Vol #, and page #: 33, page 248
Conducting laboratory and location: HMR, Cedex, France
Date of study initiation: In vivo portions of these studies were reported as 96/8486/TX, 96/9255/TX, 96/8485/TX and 14869 TCC, respectively.

Six week old rats and 10 month old dogs were given HMR 3647 orally at 50, 150 or 300 mg/kg/d (rats) or 20, 50 or 150 mg/kg/d (dogs) for 1 and 3 months. The comparator drug was Azithromycin at 50, 100 or 200 mg/kg/d for 1 month.

Transmission electron microscopy was performed on liver sections from the study animals. Examination revealed secondary lysosomes with concentric multilamellar inclusions. Results:

1) 1 month rat study: At 300 mg/kg/d, the structures were found in bile duct cells in males and in bile duct cells and hepatocytes in females. At 150 mg/kg/d, only the females showed lysosomes in bile duct cells. Males had no analogous structures. Azithromycin induced these structures at doses of ≥50 mg/kg/d.

2) 3 month rat study: At 150 mg/kg/d, females showed lysosomes in bile duct cells but not in hepatocytes.

The sponsor concluded that the intensity and duration of the changes were not aggravated with time.

3) 1 month dog study: At 300 mg/kg/d, lysosomal structures were seen in both sexes primarily in hepatocytes and, to a lesser extent, in bile duct cells.

4) 3 month dog study: Same findings as the 1 month study but lysosomes were primarily in bile duct cells.

Therefore, bile duct cells were primarily affected in rats whereas hepatocytes were mainly involved in dogs. Female rats appeared more sensitive than male rats.

The sponsor concluded that the findings were consistent with 'compound-induced phospholipidosis', encountered with macrolide antibiotics. They found the changes induced by HMR 3647 to be less pronounced than those induced by azithromycin, and were of not toxicologic consequence.

Study Title: Nephrotoxicity Study of HMR 3647 After Single Oral Administration in the Rabbit
Study No: 97/9744/TX
Vol #, and page #: 34, page 002
Conducting laboratory and location: HMR, Cedex, France
Date of study initiation: 6/10/97
GLP compliance: No
Methods:
Dosing:
- species/strain: New Zealand White rabbits
- #/sex/group or time point: 3 females
- age: 10 weeks
- weight: 1.8- 2.3 kg
- dosage groups in administered units: 0, 100, 200 or 400 mg/kg as a single dose and a group on water restriction for 4 days given 1 dose at 400 mg/kg
- route, form, volume, and infusion rate: Gavage

Drug, lot: Batch #14
Formulation/vehicle: 0.5% methylcellulose
Observations and times:

- Clinical signs: Daily
- Body weights: Pre-dosing and at termination
- Hematology: Days –1, 1, 2 and 3
- Clinical chemistry: Same as hematology
- Urinalysis: Same as hematology
- Gross pathology: All animals
- Organs weighed: Kidney and liver only
- Histopathology: Kidney and liver only

Results:

- Clinical signs: No significant treatment-related signs were reported
- Body weights: No significant treatment-related differences from controls were noted
- Hematology: No significant treatment-related findings were reported
- Clinical chemistry: No significant differences from controls were noted
- Urinalysis: No significant differences from controls were noted
- Organ Weights: No significant differences from controls were noted
- Gross pathology: No significant differences from controls were noted
- Histopathology: No significant differences from controls were noted

Key Study Findings: The sponsor concluded that HMR 3647 did not induce renal impairment under the conditions of this study.

Study Title: Nephrotoxicity Study of HMR 3647 Given Orally in a Single Dose to Renally Impaired Wistar Rats
Study No: — 78-80
Vol #, and page #: 34, page 95
Conducting laboratory and location: —
Date of study initiation: 7/17/98
GLP compliance: Japanese GLP
QA- Report: Yes
Methods: Study was conducted to evaluate the influence of HMR 3647 on renal failure.
Dosing:

- species/strain: Renally impaired rats (given glycerol [G] and furosemide [F]) and normal Wistar rats
- #/sex/group or time point: 6 for toxicity portion, 9 for PK portion, 6 for the supplementary study
- age: 7 weeks at study initiation
- weight: Males: 166- 189 g; females: 119-136 g
- dosage groups in administered units: HMR 3647 at 400 or 800 mg/kg p.o., G at 1 mL/kg s.q., F at 50 mg/kg s.q. or CER at 1000 mg/kg i.v. for the toxicity groups. For the PK groups,
- route, form, volume, and infusion rate: HMR 3647 at 20 mL/kg, CER at 5 mL/kg, G at 2 mL/kg and F at 5 mL/kg

Drug, lot: Batch #17. The positive control was Cephaloridine [CER]
Formulation/vehicle: 0.5% methylcellulose
Observations and times:

- Clinical signs: Once prior to dosing, 3x/day on day of dosing and twice on Day 2
- Body weights: Once prior to dosing, daily thereafter
- Hematology: Not performed
- Clinical chemistry: At study termination for toxicity study animals
- Urinalysis: Samples taken prior to dosing, up to 24 hours post-dosing and 24-48 hours post-dosing
- Gross pathology: All animals in toxicity groups
- Organs weighed: None
- Histopathology: Kidneys from all toxicity group animals, and tissues with gross abnormalities from occasional animals
- Toxicokinetics: Samples were taken from 3/sex at 0.5, 1, 3, 6, and 24 hours after dosing.

Results:

- Clinical signs: Three males and 1 female from the CER group and 3 males from the CER+G+F group died prematurely.

The HMR 3647 groups showed urine staining and low water consumption compared to controls.

The HMR+G+F groups showed decreased spontaneous activity on the day of dosing, and urine staining thereafter. One female at 400 mg/kg HMR 3647 had reddish urine. These animals showed lower water consumption than controls.

The CER animals showed decreased spontaneous activity.

The CER+G+F animals showed decreased spontaneous activity, hypothermia, lacrimation, pale skin and reddish urine in most animals.

- Body weights: No significant differences between groups were appreciated.
- Clinical chemistry: Day of dosing:

1) G+F group: Increased urine volume, low urine specific gravity, low NAG and creatinine values in males; high LDH, ALP and γGTP in females.
2) HMR 3647 groups: Low urine volume in 400 mg/kg males, high ALP, NAG and creatinine values in all 400 mg/kg animals. At 800 mg/kg, low urine volume, high ALP, γGTP, NAG and LAP values were reported.
3) HMR 3647+G+F group: High ALP, γGTP and NAG values with low urine volume for 400 mg/kg males, and high creatinine in 400 mg/kg females. High NAG values in the 800 mg/kg males.
4) CER+G+F group:

- Urinalysis: High occult blood was reported in the G+F group with low pH in males and high protein in females. In the HMR 3647 groups, 400 mg/kg males showed low pH as did the 800 mg/kg animals of both sexes. The HMR+G+F animals had low pH in 400 mg/kg males and 800 mg/kg animals of both sexes. High occult blood was found on Day 2 in the high dose group. These findings were comparable to those from the CER+G+F group.

- Organ Weights
- Gross pathology: No gross lesions were appreciated at necropsy other than cecal dilatation, an expected finding in rodents treated with antimicrobial agents. In the CER survivors, cecal dilatation was reported, as were discoloration of the kidney in all males and 2 females, renal enlargement and retention of red urine in the bladder.

Survivors from the CER +G+F group showed cecal dilatation, discoloration of the kidney, black content to the stomach and large intestine and 1 had black contents in the small intestine.

- Histopathology: The HMR 3647+G+F groups had regenerating renal tubules and necrosis of the proximal tubules at 800 mg/kg (females), sometimes with associated increased mitotic rates in the tubules and vacuolation of the tubules.

The CER survivors had renal cases, necrosis of the proximal tubules in 2 males and all females, and vacuolation in tubules. The premature decedents from this group had congestion, hyaline casts, necrosis of the proximal tubules and vacuolation of the proximal tubules.

All other incidental findings were comparable across groups.

Electron microscopy: Evaluations were done on kidneys from 2 males/group. In the G+F group, very slight vacuolation of proximal tubules and an increase in lysosomes was reported for 1 male.

In the HMR 3647 groups, very slight vacuolation of the proximal tubules was reported in the 400 and 800 mg/kg animals.

The CER males had vacuolation of the proximal tubules and an increase in phagosomes, vacuolation of the distal tubules and necrosis of the proximal tubules.

In the CER+G+F males, necrosis of the proximal and distal tubules and vacuolation of tubular epithelium and swelling of Bowman's epithelial cells and an increase in phagosomes in the proximal tubules were reported.

- Toxicokinetics

Key Study Findings: The sponsor concluded that one of the causes of death was acute renal failure. However, the renal failure was probably not severe enough to cause mortality as no gross changes were evident and the histologic evaluation showed only very slight changes. As animals were only given 1 dose of drug, it is difficult to determine whether 24 hours would be sufficient to elicit significant gross and histologic lesions. The sponsor considered the lesions induced by CER to be more significant than those induced by HMR 3647. They also concluded that HMR 3647 has "very little effect on the impaired kidney". The sample size is too small to determine the validity of that conclusion.

Study Title: Serum Concentrations of HMR 3647 Observed During the Study Entitled: Nephrotoxicity Study of Dose in Renally Impaired Wistar Rats (reviewed immediately above)
Study No: 98/10642
Vol #, and page #: 36, page 2
Conducting laboratory and location: HMR, Cedex, France

- Toxicokinetics: Samples were taken at 0.5, 1, 3, 6, and 24 hours after dosing. One sample (at 3 hours) or 2 samples (at 0.5-6 or 1-24 hours) were taken per animal with 3/dose/time point. The limit of quantitation of the assay was 0.05 mg/L.

Results:

- Toxicokinetics: Since many of the Group 6 and 7 animals died prematurely, the sponsor treated another group "according to a similar protocol to obtain blood samples at 6 and 24 hours".

Parameter Males	Time	Group 4 (nrl) @ 400 mg/kg	Group 5 (nrl) @ 800 mg/kg	Group 6 (impaired) @ 400 mg/kg	Group 7 (impaired) @ 800 mg/kg
Mean Serum Concentration Cmax	0.5 1 3 6 24	2.3 2.64 5.7 7.7 0.94	3.45 8.1 11.9 16.4 2.44	0.83 2.68 1.8 2.12 1.96	1.73 3.76 6.9 7.6 7.54
AUC (0-24) mg/L/hr		107.8	236.3	48.2	170.2
Females					
Mean Serum Concentration	0.5 1 3 6 24	2.11 3.9 6.74 3.8 2.72	4.2 10.67 14.16 14.7 3.13	0.67 1.03 2.41 4.5 1.83	2.24 4.9 7.3 4.13 6.0
AUC (0-24) mg/L/hr		87.52	233.61	71.23	122.89

Key Study Findings: In normal rats, the serum levels increased in proportion to dose, while in impaired males, levels increased at greater than dose proportionality. The AUCs for impaired rats were significantly lower than for normal rats and the exposure was underestimated compared to normal rats.

Study Title: Preliminary Two Week Ototoxicity Study with HMR 3647 by Oral Route (Gavage) to Rats
Study No: 17920 TSR
Vol #, and page #: 36, page 30
Conducting laboratory and location: [redacted]
Date of study initiation: 12/7/98
GLP compliance: Conducted in compliance but was neither audited by the QA department nor the final study reviewed.
Methods:
Dosing:

- species/strain: Sprague-Dawley rats
- #/sex/group or time point: 5
- age: 6 weeks of age

- weight: 167- 189 gms for males, 130- 150 gms for females
- dosage groups in administered units: 150 mg/kg/d for 2 weeks. Kanamycin was given at 100 or 300 mg/kg/d and controls were given 0.5% methylcellulose
- route, form, volume, and infusion rate: Gavage as 3 mL/kg/d for the HMR 3647, and i.m. for Kanamycin. It is not clear how the toxicity profiles compare between the routes of administration.

Drug, lot: 8N0364B for HMR 3647; 38H0885 for Kanamycin
Formulation/vehicle: 0.5% methylcellulose
Observations and times:

- Clinical signs: Daily
- Body weights: Pre-dosing, Day 1 and twice/week until termination
- Food consumption: Pre-dosing, Day 1 and twice/week until termination
- Hematology: Controls and Kanamycin-treated animals only at study termination
- Clinical chemistry: Controls and Kanamycin-treated animals only at study termination
- Gross pathology: All animals at study termination
- Histopathology: Gross lesions and inner ear (after instillation of formalin through the perforated eardrum) only from all animals at study termination. Liver and kidney from control and Kanamycin-treated animals only.
- Toxicokinetics: Not performed
- Other: Brainstem Evoked Responses Audiometry (BERA) on 3/sex/group pre-dosing and at the end of the study.

Results:

- Clinical signs: Ptyalism was reported in the HMR 3647-treated animals, supposedly due to the bitter taste of the test article.
- Body weights: Body weight gains were reduced in the 300 mg Kanamycin animals (-16% in males, -10% in females). No other significant differences from controls were noted (HMR 3647-treated females- -7% compared to controls).
- Food consumption: Reduced in the 300 mg Kanamycin-treated animals (-11% in males, -8% in females).
- Hematology: No significant differences from controls were noted for the Kanamycin-treated animals.
- Clinical chemistry: Lower K+ and higher SUN (up to 44%) and creatinine (up to 33%) were noted in the 300 mg Kanamycin-treated animals.
- Gross pathology: Pale kidneys were reported for the Kanamycin-treated animals. Distended cecae were reported in the HMR 3647-treated animals
- Histopathology: No inner ear effects were found with any of the administered solutions. Renal tubular basophilia, necrosis, tubular dilatation and mononuclear cell infiltrates were reported for both Kanamycin-treated groups with a dose-related increase in incidence and severity.
- BERA results: Higher dose-related mean auditory thresholds were reported for the Kanamycin-treated animals (positive controls). No significant differences from controls were noted in the HMR 3647-treated animals.

Key Study Findings: Under the conditions of this study, no ototoxicity was elicited by treatment with HMR 3647 at 150 mg/kg/d

Study Title: <u>Four Week Ototoxicity Study with HMR 3647 By Oral Route (Gavage) to Rats</u>
Study No: 18116 TSR
Vol #, and page #: 37, page 3
Conducting laboratory and location:
Date of study initiation: 1/19/99
GLP compliance: Yes
QA- Report: Yes
Methods:
Dosing:

- species/strain: Sprague-Dawley rats
- #/sex/group or time point: 10
- age: 6 weeks of age
- weight: Mean 190 gms for males, 137 gms for females
- dosage groups in administered units: 0, 50 or 150 mg/kg/d of HMR 3647 or 100 mg/kg/d of Kanamycin

- route, form, volume, and infusion rate: HMR 3647 by gavage (10 mL/kg/d); Kanamycin by i.m. injection (2 mL/kg/d). It is not clear how the i.m. route of administration is comparable to the oral route of administration with respect to comparative toxicity. Kanamycin is not orally absorbed.
Drug, lot: HMR 3647, 8N0364B; Kanamycin, 38H0885
Formulation/vehicle: 0.5% methylcellulose
Observations and times:
- Clinical signs: Twice daily
- Body weights: Pre-dosing, on Day 1, and twice/week until study termination
- Food consumption: Twice/week
- Hematology: At study termination
- Clinical chemistry: At study termination
- Gross pathology: All animals
- Organs weighed: Adrenals, heart, kidneys, liver, spleen, thymus
- Histopathology: Gross lesions, inner ears after formalin injection through the perforated tympanum, kidneys, and liver were examined histologically from control, Kanamycin and 150 mg/kg/d animals only. Gross lesions and inner ears from low dose HMR 3647 animals. The following tissues were preserved but not examined: adrenals, cecae, colon, duodenum, heart, ileum, jejunum, lungs, esophagus, spleen, stomach and thymus
- Toxicokinetics: Not performed
- Other: BERA pre-dosing and at study termination (Day 24 or 25)

Results:

- Clinical signs: As in the previously reviewed rat studies, ptyalism was reported for the HMR 3647-treated animals (5/10 males, 3/10 females at 50 mg/kg/d and all high dose animals). The sponsor attributed it to the bitter taste of the test article.

- Body weights: Higher weight gains were reported in the 150 mg/kg/d HMR 3647-treated females (+23%).

- Food consumption: No treatment-related effects were reported except that the high dose HMR 3647 females consumed statistically significantly higher amounts (5%) than controls. This finding is not considered biologically significant.

- Hematology: No biologically significant treatment-related effects were reported.

- Clinical chemistry: SUNs were increased in Kanamycin-treated males. ALT levels were increased in males (1.6x) and females (2.9x) treated with HMR 3647 at 150 mg/kg/d. At 50 mg/kg/d, 1/10 females showed an increased ALT value (1.6x). The sponsor did not consider the findings in the 50 mg/kg/d females significant as no histologic correlates were found. Therefore, it is difficult to assign significance to the same amount of change seen in 1/10 male at 150 mg/kg/d. Additionally, serum calcium was increased in all HMR 3647-treated animals (<10%).

- Organ Weights: No significant treatment-related effects were reported. Absolute and relative liver weights were increased in the 150 mg/kg/d HMR 3647-treated animals (males: 9%; females: 10%). No histologic correlates were reported. Thymic weights were decreased in the 150 mg/kg/d HMR-3647-treated males.

- Gross pathology: Cecae were distended in 1 female treated with Kanamycin, 4/10 males and 3/10 females treated with HMR 3647 at 50 mg/kg/d and all HMR-3647-treated animals at 150 mg/kg/d. This is an expected finding with antimicrobial treatment in rodents.

- Histopathology: No inner ear lesions were found. It was expected to find lesions in the Kanamycin – treated animals. The sponsor suggested that it could be due to the method of administration or the low dose.

Kanamycin-treated animals (all males, 3/10 females) had slight to moderate renal epithelial degeneration/necrosis with tubular basophilia and/or tubular dilatation. The incidence of tubular basophilia and/or dilatation in the HMR 3647-treated animals was higher than in controls, but the severity was comparable to controls.

- BERA results: Higher mean auditory thresholds were noted in Kanamycin-treated animals (28 dB) vs. controls (12 dB). The parametric analysis showed a highly significant dose effect with the estimated odds ratio >40. No statistically significant effects were noted in the HMR-3647-treated animals. However, an increase of 10 dB was noted in 6/20 and 3/20 for the 50 and 150 mg/kg/d HMR 3647 groups, respectively.

Key Study Findings: No significant aggravation of the auditory threshold was shown with HMR 3647 treatment.

Study Title: **Antigenicity Study of HMR 3647 in Guinea Pigs: Active Systemic Anaphylaxis and Passive Cutaneous Anaphylaxis Tests**
Study No: 016631
Vol #, and page #: 38, page 97
Conducting laboratory and location: Nippon Hoechst Marion Roussel Limited, Shiga, Japan
Date of study initiation: 3/10/97
GLP compliance: Yes
QA- Report: Yes
Methods:
Dosing:

- species/strain: Male Hartley guinea pigs
- #/sex/group or time point: 5 for test article and ovalbumin, 3for the vehicle control
- age: 5 weeks old
- weight: 324-425 gm
- dosage groups in administered units: 1 or 4 mg/kg orally or 16 mg/kg subcutaneously in combination with adjuvant. Positive controls received 2 mg ovalbumin with adjuvant.

Drug, lot: Batch 9
Formulation/vehicle: 0.5% methylcellulose for the oral dosing; 0.5% physiologic saline for subcutaneous dosing. After starting the sensitization, the sponsor determined that the concentration of the 0.2% HMR 3647 solution was inadequate, so the animals were euthanized and this portion of the study was re-initiated with new animals.
Observations and times:

- Clinical signs: Once/week for general condition and at study termination
- Body weights: Once/week and at study termination
- Food consumption: Not performed
- Gross necropsy: All thoracic and abdominal organs were examined for abnormalities.
- Other: Animals were examined for anaphylactic shock at 15 minutes, and 30 and 60 minutes post-challenge.

Results:

- Clinical signs: No significant abnormalities were reported for the 1 mg/kg HMR 3647 group. At the higher doses, soft feces were reported at 4 mg/kg, and skin crusts with alopecia were reported in the 16 mg/kg group. The ovalbumin-treated animals showed crusts and alopecia at the injection sites and one had skin ulceration. Four controls had alopecia or crusts at the injection sites.
- Body weights: Weight gains were decreased in the 1 and 16 mg/kg HMR 3647 groups when compared to controls. "Transiently decreased body weight was observed in the group administered 4 mg/kg of HMR 3647."
- Gross pathology: Cecal distention was reported in 2 guinea pigs given 1 mg/kg HMR 3647, and all animals given 4 mg/kg. Ovalbumin decedents showed distention of the lung and discoloration of the parenchyma. Similar findings were reported for 1 animal at 1 mg/kg and 1 animal at 16 mg/kg.
- Anaphylaxis: No signs were noted in the HMR 3647-treated animals but all 5 animals sensitized with ovalbumin became shocky and died.
- PCA: Antisera from HMR-3647 and methylcellulose-treated animals showed no PCA reaction. All ovalbumin-treated animals were positive with titers >16, 384.

Key Study Findings: HMR 3647 elicited neither passive cutaneous anaphylaxis nor active systemic anaphylaxis. The positive controls (ovalbumin) responded appropriately. It is reasonable to assume that HMR 3647 is not antigenic under the conditions of this study.

Study Title: **Toxicokinetics of HMR 3647 during the study entitled: Reproductive toxicology results (in vivo and litter data) of the effects of HMR 3647 administered by oral route to pregnant rats for a toxicokinetic study**
Study No: 97/9826/CN
Vol #, and page #: 41, page 198
Conducting laboratory and location: HMR, Cedex, France
Date of study initiation: 5/27/97

GLP compliance: Yes
QA Report: Yes
Methods: Main study was in pregnant rats treated Gestation Days 6-17 once/day with 50 or 300 mg/kg of HMR 3647 or the same doses only on Day 6 of gestation (Study 97/9472/TX)
Dosing:

- species/strain: Sprague-Dawley rats
- #/sex/group or time point: 10 pregnant females

- Toxicokinetics: Samples were taken from the singly dosed animals on Day 6 at 0.5, 3, and 24 hours post-dosing, and on Day 17 for the repeat dose groups at the same timepoints. Two samples/each rat were taken. They were evaluated by HPLC and fluorescence with a limit of quantitation of —

Results:

- Toxicokinetics: Two 50 mg single dose and one 50 mg repeat dose animals were not pregnant so their data were not included in the analysis.

Mean PK Parameters for Pregnant Rats Treated with HMR 3647 (N= 5)

Parameter	Day	Time (hr)	50 mg/kg	300 mg/kg
Plasma [] (mg/L)	6	0.5	7.64	2.85
		3	2.42	7.40
		24	0.006	1.74
	17	0.5	0.313	5.2
		3	0.631	7.5
		24	LOQ	2.32
AUC (mg.h/L)	6		29.64	109.52
	17		7.92	121.19

Variability between animals was rather high, especially at the 24 hour time point. There was a marked decrease in AUC from Day 6 to Day 17 for the 50 mg/kg group (2.5x). It is not clear why that happened. The increase in AUC between doses was not dose proportional; it was lower than the increase in dose on Day 6 and higher than the increase on Day 17.

Study Title: **Toxicokinetics of HMR 3647 during the study entitled: Study for effects of HMR 3647 on embryo-fetal development by oral administration (gavage) in rabbits**
Study No: 97/9881/CN
Vol #, and page #: 43, page 2
Conducting laboratory and location: HMR, Cedex, France
Date of study initiation:
GLP compliance: Yes
QA- Report: Yes
Methods: The in vivo portion of this study was Report #15277
Dosing:

- species/strain: New Zealand White rabbits
- #/sex/group or time point: 4
- dosage groups in administered units: 20, 60 or 180 mg/kg/d from Gestation Day 6-18

- Toxicokinetics: Samples were taken on Day 6 and Day 18 at pre-dosing, and 0.5, 2, 4, 8 and 24 hours post-dosing. Samples were assayed by HPLC and fluorescence with the limit of quantitation at —

Results:

- Toxicokinetics:

Mean PK Parameters of Pregnant Rabbits Treated with HMR 3647 (N=4)

Parameter	Day	20 mg/kg/d	60 mg/kg/d	180 mg/kg/d
Cmax (mg/L)	6	4.87	15.4	19.4
	18	1.06	7.2	6.1
Tmax (hr)	6	0.5-2	0.5-2	0.5-4
	18	0.5-2	0.5	0.5-2
AUC (mg.h/L)	6	22.7	109	264
	18	5.9	17.0	16.5

As in the rat study, inter animal variability was large and there were large decreases in Cmax and AUC between the test days. Increases in AUC were approximately proportional to dose between 20 and 60 mg/kg, but were much lower than dose proportional between 60 and 180 mg/kg.

Study Title: **Toxicokinetics of HMR 3647 during the study entitled: Study for effects of HMR 3647 on embryo-fetal development by oral administration in rabbits (Himalayan)- Study #97.0860**
Study No: 98/10207/CN
Vol #, and page #: 43, page 292
Conducting laboratory and location: HMR, Cedex, France
Date of study initiation:
GLP compliance: Yes
QA- Report: Yes
Methods: The in vivo portion was reported as #97/0860
Dosing:

- species/strain: Himalayan rabbits
- #/sex/group or time point: 24 (20 main study animals, 4 for toxicokinetics)
- dosage groups in administered units: 20, 60 or 180 mg/kg/d from Day 6-18 of gestation and in the fetus and the dam on Day 29

Observations and times:

- Toxicokinetics: Samples were taken from the dams on Day 6 pre-dosing and 4 and 24 hours post-dosing and on Day 18 at 4 and 24 hours post-dosing. Samples were taken from pups on Day 29 at 4 hours post-dosing. Samples were assayed by HPLC and fluorescence with a limit of quantification of [redacted]

Results:

- Toxicokinetics: Samples were taken from controls 4 hours post-dosing and the concentrations were between [redacted]. This invalidates the study, as the controls should have no HMR 3647 in their plasma.

Mean Plasma Concentrations in Rabbits Treated with HMR 3647 (N=4)

Animal	Parameter	Day	20 mg/kg	60 mg/kg	180 mg/kg
Mated ♀	Co	6	0.51	0.146	1.6
Mated ♀	C4hr	6	0.376	4.0	17.0
		18	0.135	1.27	20.9
Fetus		29	0.011	0.36	0.46
Mated ♀	C24h	6	0.017	0.13	0.234
		18	0.19	0.14	1.9

Key Study Findings: This is not considered a valid study as the controls had detectable drug levels.

Study Title: **Toxicokinetics of HMR 3647 during the study entitled: Study for effect of HMR 3647 administered by oral route (gavage) on pre- and post-natal development including maternal function in rats- Study #16218RSR**
Study No: 98/10609/CN
Vol #, and page #: 46, page 3
Conducting laboratory and location: HMR, Cedex, France
OECD GLP compliance: Yes
QA- Report: Yes
Methods: The in vivo portion of this study was reported as 16218 RSR
Dosing:

- species/strain: Sprague-Dawley rats
- #/sex/group or time point: 25 for main study and 6 for toxicokinetics. The first 3 were used for the placental transfer portion (treated from Gestation Day 6-20). The second 3 were used for the milk study (treated from Gestation Day 6-Lactation Day 10).
- dosage groups in administered units: 0, 50, 125 or 200 mg/kg/d from Gestation Day 6- Lactation Day 10

Drug, lot: Batch 22
Observations and times:

- Toxicokinetics: Samples were taken on Gestation Day 6 at 1, 6 and 24 hours post-dosing from 3 animals/group, on Gestation Day 20 at 6 hours after dosing in dams and feti, and on Lactation Day 10 at 1, 6 and 24 hours post-dosing in 3 animals.

Results:

Mean Plasma and Milk Concentrations of HMR 3647 in Rats Treated with HMR 3647

Day	Time	# of doses	Medium	50 mg/kg/d	125 mg/kg/d	200 mg/kg/d
G6	1	1	Dam plasma	0.219	0.46	0.80
	6			1.01	1.93	2.93
	24			LOQ	0.290	0.75
G20	6	15	Dam plasma	1.56	3.35	5.20
			Fetal plasma	0.148	0.76	2.56
L10	1	26/27	Dam plasma	0.52	1.01	1.4
	6			0.265	1.79	4.27
	24			0.019 (2/3 LOQ)	0.047	0.50
L10	1	26/27	Milk	1.24	2.39	5.01
	6			1.23	9.25	23.39
	24			0.084	0.52	4.06

Key Study Findings: Placental transfer occurs and increases with increasing dose. Appearance of test substance in the milk at 3.3- 6.6x that in the plasma presumes exposure of the neonate. Interanimal variability was high. Increased concentrations in the feti were > dose proportional while in the dams, the increase was ~ dose proportional.

Study Title: In Vitro Mammalian Chromosome Aberration Test with HMR 3647 in Cultured Human Lymphocytes with Monkey Liver S9
Study No: 18861 MLH
Study Type: Chromosomal aberration with monkey S9 in vitro to produce the primary metabolite in man
Volume # and Page #: 47, page 2
Conducting Laboratory:
Date of Study Initiation/completion: First experiment: 7/21/99; second experiment: 8/11/99
GLP Compliance: Yes
QA- Reports: Yes
Drug Lot Number: Batch 43
Study Endpoint: Chromosome aberrations

Methodology:

- Strains/Species/Cell line: Cultured human lymphocytes
- Dose Selection Criteria:
  - Basis of dose selection: Study 14722 MLH
- Metabolic Activation System: S9 from male cynomolgus monkey liver microsomal fraction. However, no historical data were available for the monkey liver S9 fraction so it is uncertain how valid this activation was or how it extrapolates to the human treated with HMR 3647.
- Controls:
  - Vehicle: DMSO
  - Negative Controls: None
  - Positive Controls: Cyclophosphamide at 50 μg/mL for the 20 hour harvest times, none for the 44 hour harvests. For the positive control cultures, S9 fraction from rat liver was used. This invalidates these cultures as positive controls for the study as all other cultures were activated with monkey S9.
- Exposure Conditions:
  - Incubation and sampling times: 1st study: 3hours in a 5% C02 atmosphere at 37°C with cultures harvested 20 hours after beginning incubation. 2nd study: Same incubation and treatment with harvest after 20 and 44 hours.
- Doses used in definitive study: 15.625, 31.25, 62.5, 125, 250, 500, 750 and 100 μg/mL (1st expt.)
  2nd study: 15.625, 31.25, 62.5, 125, 250 and 375 μg/mL at 20 hour harvest and 125, 250, 375, 500, 750 and 1000 μg/mL at 44 hour harvest.

- Study design:
- Analysis:
  - No. slides/plates/replicates/animals analyzed: 2 cultures/dose level
  - Counting method: Giemsa stained fixed cells on glass slides
  - Cytotoxic endpoints: # of cells in mitosis/number of cells examined (1000)
  - Genetic toxicity endpoints/results: Metaphase analysis of 200 metaphases/dose level with 100 metaphases/culture. Total number of structural aberrations and the frequencies were reported
  - Statistical methods:
- Criteria for Positive Results: Reproducible and statistically significant increase in the frequency of cells with chromosomal aberrations (excluding gaps) compared to vehicle controls (p=0.05 as the lowest level of significance).

Results:

- Study Validity: The positive (with rat S9 activation) and control cultures performed as expected. No increase in structural chromosomal aberrations was induced when monkey S9 fraction was used instead of rat S9. No historical data were available for the monkey liver S9 fraction so it is uncertain how valid this activation was or how it extrapolates to the human treated with HMR 3647. The sponsor analyzed the treatment medium and concluded that: "Metabolism of HMR 3647, even though it was low, did occur during the experiment." The range of metabolite concentrations was reportedly between — and — mg/L in the first experiment and between —and — .ng/L during the second experiment

- Study Outcome: A significant decrease in the mitotic index was found in study #1 at doses ≥ 125 μg/mL (45-64%). At 500 μg/mL, insufficient metaphases were found for analysis. At 250 μg/mL, a 45% decrease in the mitotic index was reported. No increase in the frequency of aberrations was noted.

In study #2, a moderate decrease in the mitotic index was reported at doses ≥250 μg/mL (20 hour harvest). In this portion of the study, the 375 μg/mL dose induced a 64% decrease in the mitotic index. The second harvest was performed (in this segment, the first harvest did not show a decrease in the mitotic index at doses up to 375 μg/mL). A marked to total decrease in mitotic index was found from 500 to 1000 μg/mL. The 500 μg/mL dose elicited a 41% decrease in the mitotic index. At 750 μg/mL, a 85% decrease in the mitotic index was reported so this dose was considered too toxic. No increase in the frequency of chromosomal aberrations was reported.

Summary: The sponsor concluded that: "Even though the monkey S9 did not appear to have metabolized CPA [cyclophosphamide] sufficiently to induce chromosomal aberrations, the test system was exposed to HMR 3647 and its metabolite RU76363. It can therefore be concluded that under the experimental conditions followed by this study, HMR 3647 did not show any clastogenic potential in cultured lymphocytes, with S9 mix using monkey liver S9." However, as the positive controls were not handled in the same manner as the other cultures, it does not appear that this study was valid to determine the clastogenic potential of the metabolite as stated in the objective for the study.

Study Title: <u>In Vitro Mammalian Cell Gene Mutation Test with HMR 3647 in L5178Y TK± Mouse Lymphoma Cells without and with Monkey Liver S9</u>
Study No: 18860 MLH
Volume # and Page #: 47, page 81
Conducting Laboratory: —
Date of Study Initiation/completion:
GLP Compliance: Yes
QA- Reports: Yes
Drug Lot Number: Batch 43
Study Endpoint: Mutations at the thymidine kinase locus in mouse lymphoma cells after metabolic activation with monkey liver S9

Methodology:

- Strains/Species/Cell line: L5178Y cells
- Dose Selection Criteria:

- Basis of dose selection: Study 14108 MLY
- Metabolic Activation System: S9 from male cynomolgus monkey liver microsomal fraction. However, no historical data were available for the monkey liver S9 fraction so it is uncertain how valid this activation was or how it extrapolates to the human treated with HMR 3647.

- Controls:
  - Vehicle: DMSO
  - Negative Controls:
  - Positive Controls: Without S9: methylmethane sulfonate (MMS) at 5 μg/mL; with S9: cyclophosphamide (CPA) at 3 μg/mL. In the first experiment, rat S9 was used for vehicle and positive controls. In the second experiment, monkey S9 fraction was used and no historical data were provided for this metabolic activator. The sponsor considered the results to be comparable to those achieved with rat S9 and "no clear increase in the mutation frequency was induced with CPA when monkey S9 was used instead of rat S9." This does not seem to be a reasonable conclusion as the positive controls were not positive.

- Exposure Conditions:
  - Incubation and sampling times: Without S9 fraction, incubation was for 24 hours. With S9, incubation was for 3 or 24 hours.
  - Doses used in definitive study: Without S9: 31.25, 62.5, 125, 250, 500, 750 and 1000 μg/mL. With S9, 62.5, 125, 250, 500, 750 and 1000 μg/mL for the first experiment; 31.25, 62.5, 125, 250 and 500 μg/mL for the second experiment
  - Study design:
- Analysis:
  - Counting method: Cells were counted manually
  - Cytotoxic endpoints: Measuring cloning efficiency immediately after treatment. 1.6 cells/well with 2 plate/dose were used.
  - Genetic toxicity endpoints/results: 2 plates/dose level were used to select the trifluorothymidine resistant mutant cells and small and large colonies were differentiated.
  - Statistical methods: Poisson distribution for cloning efficiency and survival relative to vehicle controls.
- Criteria for Positive Results: A reproducible 2x increase in mutant frequency compared to controls and/or evidence of a dose-response relationship.

Results:
- Study Validity:
- Study Outcome: Without S9, toxicity was marked at doses ≥ 125 μg/mL (87-100% decrease in cloning efficiency). No increases in mutation frequency were appreciated.

With S9, the first study showed marked toxicity at doses ≥ 125 μg/mL and no increases in mutation frequency were appreciated. The second experiment with S9 had mutation frequencies in the controls >the stated criteria (60-250x 10^{-6}). "Even though not valid, this assay showed no increase in the mutation frequency was induced in the presence of the test substance." Insufficient monkey S9 was available to repeat this experiment.

Summary: This is not considered a valid assay.

OVERALL SUMMARY AND EVALUATION:

Safety Evaluation:
The primary concern is the cardiovascular toxicity (including prolongation of QTc) noted in the dog and compound-induced phospholipidosis in rats and dogs.

ALTs and ASTs were increased in 150 mg/kg/d rats treated orally for 13 weeks. Histologic changes were found that were considered to be due to phospholipidosis, reported with other antimicrobial agents. In the 6 month rat study, increased ALTs and ASTs were reported for animals treated orally at 20, 50 or 150 mg/kg/d. These findings in correlation with the histologic findings were consistent with a compound-induced phospholipidosis, but were more

pronounced and in more tissues than in the 3 month study. Cmax and AUC increased between the 30 day test point and the 163 day test point, with the AUC (0-24) being ~5x higher for females than males. Bile duct cells were primarily affected (secondary lysosomes with multilamellar inclusions on electron microscopy) in rats, while hepatocytes were primarily affected in dogs.

ALTs, and ASTs were increased in dogs treated orally at 150 mg/kg/d for 13 weeks. Additionally, loss of retinal reflectivity was noted in these animals.

ALTs, ASTs and LDH were increased dose proportionally in cynomolgus monkeys treated orally for 15 days at 100 mg/kg/d. In the 28 day monkey study, ALTs, ASTs and bilirubins were increased at 60 and 120 mg/kg/d. Cmax and AUC increased between Days 1 and 28 by a factor of 2.

Coperfusion with ketoconazole and verapamil increased absorption 1.5x in the rat in situ intestinal model.

In the mouse, after single i.v. or oral administration, the t ½ was 1.2 hours in plasma, and the absorption and bioavailability were ~50%. In the rat after a single oral administration, the t ½ for plasma was 1.7 hours, for lung 3.4 hours, for brain 7.9 hours and for testes 10.2 hours. Radioactive drug remained in the pigmented tissues of the uveal tract for 12 months. The primary metabolite in the rat was the N-desmethyl-desosamine derivative. The renally impaired rats showed drug levels that increased greater than dose proportionally. The AUCs for impaired rats were significantly lower than for normal rats and the exposure was thus underestimated. In the dog, the t ½ was 2.3 hours, absorption was 83%, the bioavailability was 54% which indicates a moderate first pass effect. The primary metabolite in the dog was N-oxide-pyridine.

In rats treated for 5 days with HMR 3647, CYP3A levels were 5x control values, while dexamethasone pre-treatment elicited a 46% increase.

Placental transfer of drug occurred in pregnant rats and increased with increasing dose. Test article was found in the milk at 3.3-6.6x the plasma levels. Increased doses in the feti were >dose proportional while in the dams, the increase was approximately proportional.
Clinical Relevance of Safety Issues: In the rat liver microsome study (98/10225/CN), it appeared that the most likely enzyme for interaction was CYP3A, but a strong interaction was also found with CYP1A1. Thus a saturation of the CYP3A pathway might shift the metabolism to the CYP1A1 pathway.

Communication Review:

- Labeling Review (NDA): Will be addressed in a subsequent review

Reviewer signature: 15 1

cc: list
HFD-520/Orig. NDA
HFD-520/MO/Davidson/Makhene/Moledina/Albuerne
HFD-520/PT/Peters
HFD-520/Chem/Yu
HFD-520/Biopharm/Zheng
HFD-520/CSO/Cintron
Draft date (# of drafts): 1; 6/9/00

Concurrence Only:
HFD-520/DepDivDir/Gavrilovich
HFD-520/PTTeamLdr/Osterberg

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HFD 520/ [illegible]

REVIEW AND EVALUATION OF PHARMACOLOGY/TOXICOLOGY DATA:

KEY WORDS: HMR 3647, ketolide, cardiovascular study in dogs, safety pharmacology, toxicology studies, NDA review, genetic toxicology, reproductive toxicology
Reviewer Name: Terry S. Peters, D.V.M.
Division Name: Anti-Infective Drug Products
HFD #: 520
Review Completion Date: 3/13/00
IND/NDA number: NDA 21-144
Serial number/date/type of submission: 3/1/00
Information to sponsor: Yes
Sponsor (or agent): Aventis Pharmaceuticals, Kansas City, MO
Contact person: [redacted]
Manufacturer for drug substance: Hoechst Marion Roussel, Cedex, France

Drug:

Code Name: HMR3647
Generic Name: telithromycin
Trade Name: Ketek® (proposed)
Chemical Name:11,12-dideoxy-3-de[(2,6-dideoxy-3-C-methyl-3-O-methyl-alpha-L-ribo-hexo-pyranosyl)oxy]-6-O-methyl-3-oxo-12,11-[oxycarbonyl[[4-4-(3-pyridinyl)-1H-imidazol-l-yl)butyl]imino]]-erythromycin

Drug Class: Ketolide

Indication: Semisynthetic ketolide (macrolide) with reported efficacy against Gram positive bacteria, especially those resistant to erythromycin A. The mechanism of action is speculated to be by binding to two different sites on the ribosome, blocking the production of methylase. The sponsor is requesting approval for the following indications: 1) Community acquired pneumonia due to *S. pneumoniae*, including strains resistant to penicillin and erythromycin, *H. influenzae*, *H. parainfluenzae*, *M. catarrhalis*, *C. pneumoniae*, *L. pneumophila*, and/or *M. pneumoniae*,
2) Acute sinusitis due to *S. pneumoniae*, including strains resistant to penicillin and erythromycin, *H. influenzae*, *H. parainfluenzae*, *M. catarrhalis* and/or *S. aureus*
3) Tonsillitis/pharyngitis due to *S. pyogenes*

Proposed clinical protocol or Use: 800 mg/day qd administered as a 400 mg tablet for 7-10 days in CAP and 5 days for other indications.

Introduction and drug history: The study summaries below are excerpted from the IND 55283 reviews for this drug.
The safety pharmacology study data indicated that HMR 3647 has a significant adverse effect on the gastrointestinal tracts of dogs (vomition, ptyalism, hypomotility), and rats (delayed gastric emptying).

The sponsor concluded that 50 mg/kg/d was the NOEL for the 4 week oral toxicity study in rats on the basis of cecal enlargement, hepatoxicity at the mid and high doses, and phospholipidosis (a known class effect of macrolides) at the 150 and 300 mg/kg/d doses. The sponsor concluded that the NOEL for the 30 day oral toxicity study in dogs was 50 mg/kg/d based on the vomition, body weight decreases, nephrotoxicity, hepatotoxicity and the poor clinical condition.

The sponsor considers 90 mg/kg/d to be the NOEL for direct systemic toxicity for the four week intravenous study in rats. However, the hematologic and clinical chemistry findings are significant in that no significant histopathologic lesions (tail necrosis, loss of tail, etc.) were noted to support the claim that the inflammatory syndrome was due to the method of administration. From the review: "There were no treatment-related findings at any dose level. Slight hepatocellular hypertrophy was seen in 1/12 males and 3/12 females from the high dose group. Skin ulceration was found at the infusion sites of 8 high dose males, but none were found in the females." Therefore, the NOEL for this study is determined to be 10

mg/kg/d. In the four week intravenous study in dogs, in the high dose animals of both sexes, there was: vomition, slight to marked ptyalism, tremors (one male) on 15 treatment occasions.

The sponsor concluded that HMR 3647, daily for 30 days by the intravenous route, was clinically well tolerated at 10 and 30 mg/kg/d, but elicited vomiting and ptyalism in animals given 90 mg/kg/d. They set the NOAEL at 90 mg/kg/d.

Fertility study: The overall NOEL for the study is set at 50 mg/kg/d due to the reductions in fertility indices and fertilization.

In the rat embryo-fetal development study, the NOEL for maternal tolerance was 50 mg/kg/d, and for embryo-fetal development was 150 mg/kg/d.

In the rabbit embryo-fetal development study, the NOEL for effects on maternal and embryofetal toxicity was 20 mg/kg/d, and the NOAEL was 60 mg/kg/d.

SAFETY PHARMACOLOGY:

Effect of Bolus Intravenous Injection of HMR 3647 on Arterial Blood Pressure, Heart Rate and Electrocardiogram in Conscious Dogs. Comparison with Erythromycin and Clarithromycin; 97/10053/PH

This study was performed using 4 beagles/sex for the ketolide and 3/sex for the comparators. All drugs were dosed at 15 mg/kg i.v. over 1 minutes with a 3 day washout period between doses. Dogs were implanted with a telemetric device. Heart rate and corrected QT intervals were expressed every minute up to 20 minutes and statistical analysis was performed to compare results between test compounds.
Electrocardiography: Clarithromycin elicited a slight (insignificant- one dog), transient increase in diastolic blood pressure as did HMR 3647 (systolic pressure: 179 mmHg compared to 164 mmHg at the start; diastolic 109 mmHg compared to 95 mmHg at the start). Erythromycin elicited a moderate and long lasting decrease (134 mmHg compared to 158 mmHg at the start) in systolic blood pressure. This decrease was sustained for approximately 1 hour.

The maximal heart rate increase (~33%, 153 bpm vs. 115 bpm at the start) was reported with HMR 3647 peaking 2 minutes post-dosing. This change was maintained for 4 minutes. A second peak was noted after 9 minutes. Clarithromycin did not elicit any increase in heart rate. Erythromycin elicited a maximal increase (150 bpm vs. 117 bpm initially) at 8 minutes post-injection, and rate returned to baseline by 18 minutes. Therefore, the maximal rate increase after HMR 3647 treatment was higher than with the other test compounds.

An increase in corrected QT interval was reported with HMR 3647 (295 msec compared to 266 msec initial value- Bazett's formula, $p<0.01$ vs. controls [also statistically significant with Fridericia's formula), peaking after 1 minute. With Clarithromycin (maximum increase 17 msec at 20 minutes, not statistically significant) and Erythromycin (maximal increase 17 msec at 10 minutes, not statistically significant), the peak effect was noted 20 minutes following injection. The sponsor claims that "the mean effect of these products, unlike HMR 3647, was considerably smoothed, and a significant effect could not be seen." Statistical analysis showed a $p= 0.32$ for corrected QT interval using Bazett's formula.

At 300 mg/kg, HMR 3647 induces hyperlocomotion that could be indicative of an interaction with neurotransmitters, e.g., dopamine, noradrenaline, serotonin, or glutamate.
The sponsor concluded that HMR 3647 administered intravenously to mice induced transient hypothermia.
The sponsor concluded that HMR 3647, administered orally, did not protect against pentylenetetrazol-induced convulsions and mortality in mice. In fact, HMR 3647 administration appeared to hasten appearance of convulsions and death for the high dose animals when compared to controls.
The sponsor concluded that HMR 3647 possesses potent adrenolytic properties when administered intravenously in mice at doses of 15 and 30 mg/kg.

Effects of Intravenous Infusion of HMR 3647 on Blood Pressure and Heart Rate in Normotensive Pithed Rats; Report #97/9844/PH, dated November, 1997. This study was performed by [redacted] but no GLP compliance statement is included in the report.

Animals: 7 male Sprague-Dawley rats, aged 11 weeks at study initiation.

Doses administered: 0 (NaCl), 15 or 45 mg/kg i.v.

Procedure: Animals were anesthetized, pithed, and implanted with a blood pressure transducer. They were treated with test compound as a slow infusion. Blood pressure and heart rate were measured every 5 minutes during the infusion and for 35 additional minutes.

Results: Systolic blood pressure increased in a dose-dependent and time-dependent fashion in the HMR 3647-treated animals (3x and 6x control values in the 15 and 45 mg/kg groups, respectively). Pressures returned to normal within 20 minutes of completion of the infusion. Heart rates increased in a dose-dependent and time-dependent fashion in the HMR 3647-treated animals, reaching ~2.5x in the higher dose animals. Rates returned to normal by 15 minutes following completion of the infusion.

The sponsor concluded that at 15 mg/kg, HMR 3647 had no relevant effect on blood pressure and heart rate. At 45 mg/kg, HMR 3647 induced a direct positive chronotropic effect, with a moderate increase in pulsed blood pressure. These effects are comparable to those for the isolated guinea pig atria demonstrating both a chronotropic and inotropic effect of HMR 3647 at a concentration of 10-4M.

Effect of Intravenous Administration of HMR 3647 on Hemodynamic Parameters and Electrocardiogram in the Anesthetized Dogs; Report #97/9377/PH, dated December, 1997; **Histamine Concentration in Plasma Samples of the Study; Report #97/10129/AS**, dated November, 1997. **Plasma Concentrations of HMR 3647 during the Study; Report #97/9922/CN**, dated November, 1997.

Animals: 2 male and 2 female dogs, aged 11-18 months at study initiation.

Doses administered: 0 (NaCl), 5, or 15 mg/kg HMR 3647 administered i.v. once.
Procedure: Animals were anesthetized, hooked up to the recorders, and after a 30 minute stabilization period, treated in the sapheneous vein. Parameters were recorded for 60 minutes.

Results:
Arterial blood pressure: At 30 and 45 minutes post-dosing (for the 15 and 5 mg/kg groups, respectively), increased pressures were noted (+4-6 mm Hg). These changes were not considered biologically significant.

Heart rate: At both doses, rates (+10-23%) increased rapidly and reached a dose-dependent peak in 1 and 5 minutes, respectively. Heart rates returned to normal by the end of the experiment. PQ intervals increased compensatorily.

Cardiac output: Output rose in a dose-dependent and time-dependent fashion, but returned to normal within 40 minutes.

Cardiac contractility: At both doses, the output increased significantly and remained high for the remainder of the experimental period.

End-diastolic pressure: No effect was noted at the 5 mg/kg level. There was a slight decrease at 15 mg/kg, but returned to normal within 10 minutes.

Total peripheral resistance: Decreased resistance was noted with both doses, and remained significantly decreased for at least 10 minutes post-dosing.

Cardiac work: An increase in cardiac work was noted with both doses, but returned to normal within 30 minutes.

Plasma concentration of histamine: At 15 mg/kg, HMR 3647 induced a non-statistically significant increase in plasma histamine, which lasted up to 5 minutes post-infusion.

Plasma concentrations of HMR 3647: Maximum concentrations were found with both doses 8 minutes after initiating dosing. The maximum concentration was between [redacted] mg/L in the 5 mg/kg group, and between [redacted] mg/L in the high dose group.

The sponsor concluded that HMR 3647 showed a cardiac impact, when the plasma concentration exceeded 10 mg/L. The relevance of this effect has to be verified. Neither dose had any effect on stroke volume, heart rhythm, QT interval or duration, or R-R interval.

<u>In Vitro Effect of HMR 3647 on Isolated Guinea- Pig Atria</u>; **Report #96/8973/PH**, dated April, 1997.
Animals: 6 male Dunkin Hartley guinea pigs/dose

Doses used: 10-4, 10-5, or 10-6 M HMR 3647 or 5x10-8 isoprenaline as 0.1 mL/25 mL organ bath

Results: HMR 3647 had no effect on isolated guinea pig atria at the mid and high dose, but at 10-4 M, it increased contractile force ~50%. Isoprenaline increased contractile force by ~100%.
The sponsor concluded that the positive inotropic and chronotropic effects could be in relation to the slight histamine H2 receptor affinity of the test compound.

<u>Effect of Bolus Intravenous Injection of HMR 3647 on Arterial Blood Pressure, Heart Rate and Electrocardiogram in Conscious Dogs. Comparison with Erythromycin and Clarithromycin;</u>
Study No. 97/10053/PH
Date of study initiation: 10/21/97

- species/strain: beagle dogs from [redacted]
- #/sex/group or time point: 8 (4/sex) for the HMR portion, 6 for comparators
- age: 14 months
- weight: 9-13.5 kg
- dosage groups in administered units: 15 mg/kg
- route, form, volume, and infusion rate: i.v. over 1 minute, or saline (controls) with 3 day wash-out period between doses

Observations and times:

- EKG: Dogs were implanted with a telemetric device. Heart rate and corrected QT intervals were expressed every minute up to 20 minutes and statistical analysis was performed to compare results between test compounds.
- Toxicokinetics: Venous blood samples were taken immediately after dosing and 5 and 90 minutes later for determination of plasma levels of test substances.

Results:

- Electrocardiography: Clarithromycin elicited a slight (insignificant- one dog), transient increase in diastolic blood pressure as did HMR 3647 (systolic pressure: 179 mmHg compared to 164 mmHg at the start; diastolic 109 mmHg compared to 95 mmHg at the start). Erythromycin elicited a moderate and long lasting decrease (134 mmHg compared to 158 mmHg at the start) in systolic blood pressure. This decrease was sustained for approximately 1 hour.

The maximal heart rate increase (~33%, 153 bpm vs. 115 bpm at the start) was reported with HMR 3647 peaking 2 minutes post-dosing. This change was maintained for 4 minutes. A second peak was noted after 9 minutes. Clarithromycin did not elicit any increase in heart rate. Erythromycin elicited a maximal increase (150 bpm vs. 117 bpm initially) at 8 minutes post-injection, and rate returned to baseline by 18 minutes. Therefore, the maximal rate increase after HMR 3647 treatment was higher than with the other test compounds.

An increase in corrected QT interval was reported with HMR 3647 (295 msec compared to 266 msec initial value- Bazett's formula, $p<0.01$ vs. controls [also statistically significant with Fridericia's formula), peaking after 1 minute. With Clarithromycin (maximum increase 17 msec at 20 minutes, not statistically significant) and Erythromycin (maximal increase 17 msec at 10 minutes, not statistically significant), the peak effect was noted 20 minutes following injection. The sponsor claims that "the mean effect of these products, unlike HMR 3647, was considerably smoothed, and a significant effect could not be seen." Statistical analysis showed a $p= 0.32$ for corrected QT interval using Bazett's formula.

Results from this study indicate that bolus injection of HMR 3647 to conscious dogs elicited moderate modifications of cardiovascular parameters (blood pressure, heart rate and QT interval).

Clinical Relevance of Safety Issues: Concerns continue to exist for potential human adverse cardiac events with HMR 3647 administration, especially in cardiocompromised patients.

TOXICOLOGY:

Thirty-Day Toxicity Study of HMR 3647 in the Rat with A Four Week Recovery Period; Report #96/8486/TX, dated March, 1997

Animals: 15 Sprague-Dawley rats/sex/dose (control, mid, high), aged 6 weeks at study initiation. Ten animals/sex/low dose group only. Five/control, mid and high dose were retained for a 4 week observation period.

Doses administered: 0 (0.5% methylcellulose), 50, 150, or 300 mg/kg/d by gavage

No significant differences from controls were noted in feed consumption, ophthalmoscopy, or urinalyses.

Hematology: A neutrophilia was found in the mid and high dose males, and high dose females (39-60% increase). No significant differences from controls were found at the end of the recovery period.

Clinical chemistries: High dose males (15x) and females (4x) and mid dose females (2x) showed increased ALT values. Increased AST and leucine aminopeptidase were found in the high dose males and females. No significant differences from controls were found at the end of the recovery period.

Organ weight: Increased absolute (47%) and relative (53%) liver weights were noted in high dose females, with multinucleated hepatocytes found in these same animals. At the end of the recovery period, liver weights remained increased. Lungs were slightly increased in high dose animals of both sexes.

Histopathology: Hepatocellular necrosis was moderate to severe in high dose animals, but more pronounced in the males. Phospholipidosis (in bile duct, lung, ileum, mesenteric lymph nodes) was noted in mid and high dose animals. Reversibility was almost complete at the end of the recovery period.

The sponsor concluded that 50 mg/kg/d was the NOEL for this 4 week oral toxicity study in rats on the basis of cecal enlargement, hepatoxicity at the mid and high doses, and phospholipidosis (a known class effect of macrolides) at the 150 and 300 mg/kg/d doses.

Toxicokinetics of HMR 3647 in the Sprague-Dawley Rat After Repeated Oral Administration of HMR 3647 at Doses of 50, 150, and 300 mg/kg/d for 30 Days; Report 97/9732/CN, dated November, 1997.

Toxicokinetics Study of HMR 3647 in the Sprague-Dawley Rat After Repeated Oral Administration of HMR 3647 at Doses of 50, 150, and 300 mg/kg/d: Animal Experiment Part; Report #96/8952/TX, dated May 29, 1997.

Plasma Concentrations of HMR 3647 Measured by HPLC in Study 96/8952/TX; Report #96/9183/CN, dated July, 1997.

Toxicokinetic portion:

Animals: 18 Sprague-Dawley rats/sex/dose, aged 6 weeks at study initiation, divided into 3/sex/dose/sampling timepoint.

Doses administered: 0, 50, 150, or 300 mg/kg/d by gavage once/day for 30 days.

Procedure: Plasma concentrations were determined pre-dosing, at 0.5, 1, 3, 6, and 24 hours post-dosing on Day 1, and on Day 30. Samples were assayed by HPLC and fluorescence detection. The lower limit of detection was [redacted]

An increase in Cmax and AUC was found with the dose, but in a nonlinear fashion depending on the sex and the day. An increase in Cmax and AUC between Day 1 and Day 30 from 150 mg/kg in the female and 300 mg/kg in the male, with accumulation ratios around 3 at the high dose in both sexes.

Cmax in the 150 mg/kg female on Day 30 was 2x higher than in the male.

On Day 30, AUC (0-24) increased but not proportionally to the dose of HMR 3647 for doses between 50 and 150 mg/kg: the ratios of AUC (7.0 and 7.2 for males and females, respectively) were greater than the ratio of the dose (3). However, the AUC (0-24) appeared to increase proportionally to the dose for doses between 150 and 300 mg/kg.

Although the sponsor contends that the variability within each subgroup is generally low, with only 3 animals/sex/timepoint, a 40-80% variability provides a wide range of values.

The sponsor concluded that HMR 3647 was well tolerated by the oral route in rats at 50 and 150 mg/kg/d for 30 days.

Four Week Toxicity Study with HMR 3647 by Intravenous Route (60 minute infusion) in Rats; Report #14687 TSR, dated November, 1997.
Animals: 12 Sprague-Dawley rats/sex/dose, aged 6 weeks at study initiation.

Doses administered: 0 (NaCl), 10, 30, or 90 mg/kg/d intravenously into a tail vein, once/day for 4 weeks.

Results: No treatment-related mortality was found.

There were no treatment-related clinical signs, ophthalmoscopic findings, gross pathology, or differences from controls with respect to feed consumption or body weight.

Hematology: Leukocyte counts were higher in high dose animals (+28% in males; +59% in females), with neutrophil counts higher in mid and high dose males (~3x) and high dose females (~3x). Hemoglobin levels in mid and high dose males were reduced (-7%). Fibrinogen levels were markedly increased in the mid dose males (+58%), and both sexes (97% in males; 139% in females) in the high dose group. APTT levels were decreased (~23%) in mid and high dose males. The sponsor considered these changes to be related to an inflammatory syndrome, partially due to the mode of administration.

Clinical chemistries: High dose animals showed a lower albumin:globulin ratio (-26% in males; -33% in females) with higher beta-globulin levels than other dose groups. This is unexpected as antimicrobial administration usually leads to decreased globulin concentrations.

Organ weights: Liver weights were higher in the 90 mg/kg animals (~12%) when compared to controls.

Histopathology: There were no treatment-related findings at any dose level. Slight hepatocellular hypertrophy was seen in 1/12 males and 3/12 females from the high dose group.

The sponsor considers 90 mg/kg/d to be the NOEL for direct systemic (intravenous) toxicity for this study in rats. However, the hematologic and clinical chemistry findings are significant in that no significant histopathologic lesions were noted to support the claim that the inflammatory syndrome was due to the method of administration. Therefore, the NOEL for this study is determined to be 10 mg/kg/d.

Ten Day Oral Toxicity Study of RU66647 in the Young Dog; Report #95/7829/TX, dated December, 1996.
Animals: 3 beagles/sex/dose, aged 6 weeks at study initiation.

Doses administered: 0 (0.5% methylcellulose) or 100 mg/kg RU66647 by gavage once/d for 10 days.

Results: There were no premature deaths.

The sponsor concluded that RU66647 at 100 mg/kg/d for 10 days did not cause any significant treatment-related effects, other than increased body weight gain in the treated animals.

Thirty Day Oral Toxicity Study of HMR 3647 in the Dog; Report #96/8485/TX, dated May, 1997.
Animals: 3 beagles/sex/dose, aged 10 months at study initiation.

Doses administered: 0, 50, 150, or 300 mg/kg/d orally given daily as a capsule.

Results: One high dose female was sacrificed on Day 29 with hypotonia, hypothermia, marked dehydration and tremors. Pathology showed liver toxicity (drug-related) and renal failure (the major factor contributing to the poor condition and death of the animal).

Clinical signs: Vomition was noted in all treated animals with a dose-dependent increase in frequency and severity.
Ptyalism was noted in all mid and high dose animals. Small head and chewing movements were observed in one low dose female, 2 mid dose males, and all mid dose females, and high dose animals of both sexes.

Hypomotility was noted in one mid dose male and all mid dose females, and all high dose animals, with tremors and/or hypotonia. Dehydration was recorded in all of these animals.

Body weight: Two males (-17%) and one female (-36%) in the mid dose group lost weight. Three males (-25%) and 2 females (18%) in the high dose group lost weight.

Feed consumption: Decreased in the mid and high dose animals, especially toward the end of the study.

EKG: One mid dose animal showed sinus tachycardia on Day 1. On Day 30 (1 hour after dosing), heart rates were increased in 1 male and 2 females from the mid dose group, and 1 male and 2 females from the high dose group. The sponsor considered the elevated rates to be a stress response and questioned the relationship to treatment.

Hematology: The slight dose-dependent increase in hemoglobin concentration and PCV in the mid and high dose animals may be attributable to dehydration. An increase in fibrinogen was found in a few animals in the mid and high dose groups, but the increase was minimal.

One low dose female showed increased ALT on Day 7, but normal values at the end of the study. Mid and high dose animals showed consistently increased ALT and AST values (up to 6x control values).

On Day 30, 2 high dose males showed significant increases in creatinine (2.2x) and urea (3.6x).
There was a slight increase in iron and transferrin saturation in males and females at the high dose, and 2 males and all females in the mid dose.

Urinalyses: Granular casts were found in the urine of 3 males and 1 female from the high dose group, and 1 female from the mid dose group. These were correlated with histologic findings.

Organ weights: Thymic weights were markedly decreased in all mid and high dose animals, and one female from the low dose group.

Gross necropsy: Yellow discoloration of the kidney (cortex and/or corticomedullary junction) was noted in

2 mid dose females and one high dose male. All other macroscopic findings were considered spontaneous.

Histopathology:
Kidneys: Tubular nephropathy (marked in 2 high dose males, mild in one/sex high dose animal, and in one mid dose female) in the outer medulla, primarily affected the thick ascending Loop of Henle. Cortical tubular changes appeared to be secondary and were characterized by dilation of the distal convoluted tubules, vacuolation of the proximal and distal convoluted tubules, casts and interstitial inflammation. The sponsor contends that the renal changes are well correlated with clinical and biological signs of dehydration induced by vomiting. They state that the sequence of events is considered the major factor to explain the mortality and other kidney modifications.

Liver, gallbladder and lymph nodes: Kupffer cells, primarily centrilobular, were more prominent in the high dose animals. Histiocytosis (foamy macrophage infiltration) was noted in most of the high dose animals, and some of the mid dose animals. These changes are related to a phospholipidosis, previously described with the compound.

Thymus: Slight to marked involution in mid and high dose animals, probably due to stress.

The sponsor concluded that the NOEL for this study was 50 mg/kg/d based on the vomition, body weight decreases, nephrotoxicity, hepatotoxicity and the poor clinical condition.

Toxicokinetics: Plasma samples were collected pre-dosing, and at 0.5, 1, 2, 4, 8 and 24 hours post-dosing on Days 1 and 30.

The sponsor concluded:
1) There was high between-animals variability
2) No obvious relationship between low concentrations and vomiting
3) No apparent sex effect
4) An increase in AUCs and Cmax on Day 30 not in proportion to dose, whereas on Day 1, a marked trend to a decrease in concentrations with increasing doses was seen.
5) A marked increase in AUCs and Cmax between Day 1 and Day 30 at mid and high doses.

<u>Four Week Toxicity Study with HMR 3647 by Intravenous Route (60 minutes infusion) in Beagle Dogs</u>; **Report #14688**, dated November, 1997.

Animals: 3 beagles/sex/dose, aged 7 months at study initiation.

Doses administered: 0 (NaCl), 10, 30, or 90 mg/kg/d once/d by i.v. infusion of 60 minute duration

Results: There was no unexpected mortality during the study.
Clinical signs: In the high dose animals of both sexes, there was: vomition, slight to marked ptyalism, tremors (one male) on 15 treatment occasions.

There were no significant differences from controls in feed consumption, EKG (toxicologically insignificant increased heart rates in the high dose group), blood pressure, ophthalmoscopy, hematology, clinical chemistry, urinalyses, organ weights, gross necropsy or histopathology.
The sponsor concluded that HMR 3647, daily by intravenous route, was clinically well tolerated at 10 and 30 mg/kg/d, but elicited vomiting and ptyalism in animals given 90 mg/kg/d. They set the NOAEL at 90 mg/kg/d.

<u>Study for Effects of HMR 3647 Administered by Oral Route on Fertility and Early Embryonic Development to Implantation in Rats</u>; **Report #13973RSR**, dated June, 1997.
Animals: 24 Sprague-Dawley rats/sex/dose, and 5 satellite females for plasma drug levels at Day 7 post-coitum.

Doses administered: 0 (0.5% methylcellulose), 50, 150, or 300 mg/kg/d by gavage; in males (premating

period [29] days, mating period, and until final sacrifice) and in females (premating period [15] days, mating period, and until implantation [Day 7 post-coitum]).

Parameters evaluated: mortality, clinical signs, body weight, feed consumption, gross necropsy, epididymal sperm and testicular head sperm count (after final sacrifice), and numbers of corpora lutea, implantation sites, and dead: live fetal ratio.

Results:
All treated males and females showed ptyalism and signs of poor clinical condition and/or difficult breathing were found in one low dose male, one/sex in mid dose group, and 1 male and 3 females in the high dose group. The sponsor attributed the respiratory difficulties to possible reflux.

Male Fertility Data: Male fertility index was moderately reduced at 150 and 300 mg/kg/d dosing. Epididymal sperm and viability were not affected, but testicular head sperm count and daily sperm production were significantly reduced in these groups.

Female Fertility Data: A moderate decrease in female fertility indices was noted in the mid and high dose groups. There was a moderate decrease in the number of corpora lutea in the high dose animals.

Organ weights: A decrease in the absolute/relative weights of the male genital organs (prostate, seminal vesicle, and epididymides) was noted in the 300 mg/kg males. A similar decrease was noted in the ovaries, uteri, and vagina. "In the absence of microscopic examination, a relationship to treatment cannot be confirmed or not."

The sponsor concluded that HMR 3647 was well tolerated at 50 and 150 mg/kg/d, but poorly tolerated at 300 mg/kg/d in the rat. Male and female fertility indices were reduced at 150 and 300 mg/kg/d. Their proposed NOEL was 150 mg/kg/d for parental tolerance, 50 mg/kg/d for gametes and fertilization, and 300 mg/kg/d for mating behavior and pre-implantation/implantation. However, the overall NOEL for the study is set at 50 mg/kg/d due to the reductions in fertility indices and fertilization.

<u>Toxicokinetics of HMR 3647 During the Study #13973</u>; **Report #97/9818/CN**, dated October, 1997.
In this portion, the purpose was to evaluate the exposure to HMR 3647 in the pregnant rat on Day 7 post coitum after daily dosing of 50, 150, or 300 mg/kg/d. Samples were taken from 5 satellite females/dose on Day 7 at 1, 6, and 24 hours after dosing. The lower limit of quantitation was [redacted]

Results:

Parameter- Time (h)		50 mg/kg	150 mg/kg	300 mg/kg
Plasma [mg/L]	1	2.47	1.08	2.96
	6	0.368	2.53	5.12
	24	0.020	0.215	2.28
AUC (0-24) [mg/L/h]		5.15	34.2	88.4

The data show AUC increases non-linearly with dose, and the ratio of AUCs is higher than the ratio of the doses.

<u>Study of Effects of HMR 3647, Administered by the Oral Route, on Rat Embryo-Fetal Development (50, 150, 300 mg/kg/d)</u>; **Report #96/9298/TX**, dated November, 1997.
Animals: 25 female Sprague-Dawley rats/dose, aged 11 weeks at study initiation.

Doses administered: 0 (0.5% methylcellulose), 50, 150, or 300 mg/kg/d, by gavage, once/day from Day 6-17.

Piloerection was noted in all 300 mg/kg/d females soon after initiation of dosing (Day 8). In 4 of these animals, there was associated kyphosis. At this dose, decreased body weight gain (-12%) was reported. In the 150 mg/kg/d group, decreases compared to controls were <6%. Feed consumption was concurrently

decreased (20% and 38% for the mid and high dose animals, respectively).

Pregnancy rates were comparable across dose groups. In the mid and high dose groups, the mean uterine weights were statistically reduced (-20%), compared to controls.

The pre-implantation loss rate was slightly increased in the mid dose, but all other groups were comparable to controls. A slight dose-dependent increase in post-implantation loss rate was noted, but it did not reach statistical significance.

Fetal Parameters: Small feti: 2/controls, 1/50 mg/kg/d and 6/300 mg/kg/d. All other anomalies (soft tissue, skeletal) were within normal biological variation except for the high dose group where the incidence and severity of feti with incomplete ossification was increased when compared to controls. This group also showed bent ribs in 6/22 litters born.
The sponsor concluded that the NOEL for maternal tolerance was 50 mg/kg/d, and for embryo-fetal development was 150 mg/kg/d.

Study for Effect of HMR 3647 Administered by Oral Route (Gavage) on Pre- and Post-Natal Development Including Maternal Function in Rats; 16218 RSR
Animals: Female Sprague-Dawley rats
Doses employed: 0 (0.5% methylcellulose), 50, 125 or 200 mg/kg/d from Day 6 of pregnancy to Day 21 post-partum
Route of Administration: Gavage
Study Design: Segment III developmental study in rats
Number of animals/sex/dosing group: 25 mated females + 6 for plasma and milk levels of test article

Results:
- Clinical signs: Ptyalism was reported for F0 parents from the 3rd week of dosing (125 and 200 mg/kg/d) to the end of the study. "This clinical sign was attributed to treatment with test substance, but was not considered to represent an adverse toxic effect." As this sign was only noted for the top two dose groups, it is considered a toxic effect.

- Body weight: Mid and high dose females showed statistically significant lower body weight gain and lower feed consumption than controls. Mean body weight gain for controls was 150 g, while 125 mg/kg/d animals gained 131 g (-13%), and 200 mg/kg/d females gained only 126 g (-16%). During the lactation period, the weight gains for these groups were higher than for controls (statistical significance reached in the high dose group). A slight decrease in gain was noted in the first 3 days of dosing in the 50 mg/kg/d dams.

Fertility and Early Embryonic Development in Females
- In-life observations: Duration of pregnancy, course of parturition and litter size were comparable across groups.
- Terminal and Necroscopic evaluations: Implantation sites were comparable across dose groups. Loss of pups was 0.6 for controls, 0.7 for the 50 mg/kg/d dams, 1.4 for the 125 and 200 mg/kg/d dams.
Embryo-fetal Development
- In-life observations: The mean body weights at birth for high dose pups were lower (~10%) than controls and other dose groups. Slight growth retardation was found in this group throughout the study.

At the 200 mg/kg/d dose, neonatal survival was lower than in other dose groups. 14/323 pups died in the high dose groups vs. 3/303 for controls.

Dams: No treatment-related adverse effects were noted. Post-implantation losses were higher for the treated animals when compared to controls (5.2% for controls, and 6.5, 7.5 and 7.9 for the respective increasing dose groups).
Key findings: The sponsor concluded that the NOELs for this study in rats were as follows:
Maternal tolerance in pregnant/lactating F0 females was 50 mg/kg/d
Pre- and neo-natal development in F1 animals: 125 mg/kg/d

Post-natal development in F1 animals: 200 mg/kg/d (We find the NOEL to be 125 mg/kg/d)
Effects on fertility in F1 animals: 200 mg/kg/d.

Study for the Effects of HMR 3647 on Embryo-Fetal Development by Oral Administration (Gavage) in Rabbits; Report #15277RSL, dated November, 1997.

Animals: 24 mated New Zealand White rabbits/dose, aged 17 weeks at study initiation. Four from each group were used for determination of plasma levels of drug substance.

Doses administered: 0 (0.5% methylcellulose), 20, 60, or 180 mg/kg/d administered by gavage.

Two does aborted from the 180 mg/kg/d group.

Feed consumption was decreased and body weight loss was found in the 60 and 180 mg/kg/d groups (~8%) for the dosing period and immediately thereafter.

A moderate increase in the reduced ossification of the hyoid, reduced ossification of the talus, unossified sternebrae, presence of 13th rib, and unossified 1st metacarpal, were noted in the high dose group.

The sponsor concluded that the NOEL for effects on maternal and embryofetal toxicity was 20 mg/kg/d, and that the NOAEL was 60 mg/kg/d.

GENETIC TOXICOLOGY:
Bacterial Reverse Mutation Test with HMR 3647; Report #13611MMJ, dated March, 1997.
Tester strains: TA1535, TA1537, TA98, TA100 of *Salmonella typhimurium*; WP2uvrA of *Escherichia coli*.
The S9 fraction was prepared from a liver microsome fraction of rats induced with Aroclor 1254.

Doses tested:
First study: 0.01, 0.03, 0.1, 0.3, 1 ug/plate: TA1535 without S9 mix and TA100 with/without S9.
0.03, 0.1, 0.3, 1, 3 ug/plate: TA1535 with S9
0.1, 0.3, 1, 3, 10 ug/plate: TA1537 and TA98 without S9
0.3, 1, 2, 10, 30 ug/plate: WP2uvrA with/without S9, TA1537, and TA98 with S9

Second study: 0.03, 0.1, 0.3, 1, 3 ug/plate: all Salmonella strains without S9, TA100 with S9.
0.1, 0.3, 1, 3, 10 ug/plate: TA1535, TA1537, TA98 with S9
1, 3, 10, 30, 100 ug/plate: WP2uvrA with/without S9.

Vehicle control: DMSO
Positive controls:
Without S9: 1ug/plate NaN3 (sodium azide): TA1535, TA100
50 ug/plate 9AA: TA1537
0.5 ug/plate 2 NF: TA98
2 ug/plate ENNG: WP2uvrA

With S9: 2 ug/plate 2AM: All Salmonella strains
10 ug/plate 2AM: WP2uvrA

Results: Vehicle controls and positive controls were appropriate to prove the sensitivity of the test system.

HRM 3647 induced toxicity at all doses >1 ug/plate for the Salmonella strains, and >10 ug/plate for the E. coli strain. It did not induce any significant increases in revertants, whether in the presence/absence of S9.

The sponsor concluded that HMR 3647 did not show any mutagenic activity in this assay with S. typhimurium or E. coli.

In Vitro Mammalian Cell Gene Mutation Test with HMR 3647 in L5178Y TK +/- Mouse Lymphoma Cells; Report #14108MLY, dated March, 1997.

Procedure: HMR 3647 was tested in 2 experiments, with or without S9 (prepared from a liver microsomal fraction of rats induced with Aroclor 1254). Cells (mouse lymphoma L5178Y TK +/-) were exposed to test or control substances, in the presence/absence of S9 (final concentration 2%) for 3 hours at 37'C. Cytotoxicity was quantitated by measuring the cloning efficiency immediately after treatment, and at the end of the expression period, cell viability and mutant frequency were determined.

Test substance: Dissolved in DMSO.

15.625, 31.25, 62.5, 125, 250, and 500 ug/mL (1st experiment) without S9
31.25, 62.5, 125, 250, 500, and 750 ug/mL (2nd experiment) without S9
62.5, 125, 250, 500, 1000, and 2000 ug/mL (1st experiment) with S9
125, 250, 500, 1000, and 2000 ug/mL (2nd experiment) with S9

Positive controls: Methylmethane sulfonate (MMS) 25 ug/mL, and cyclophosphamide (CPA) 3 ug/mL

Results:

In the absence of S9, moderate to very marked cytotoxicity was observed at doses >31.25 ug/mL (relative cloning efficiency between 74% - 3% depending on dose). At lower doses, no appreciable cytotoxicity was found.

In the presence of S9, cytotoxicity was less marked (relative cloning efficiency decreased between 26% and 94% depending at doses >125 ug/mL).

The sponsor concluded that HMR 3647 did not show any mutagenic activity in the mouse lymphoma assay.

In Vitro Mammalian Chromosome Aberration Test with HMR 3647 in Cultured Human Lymphocytes; Report #14722MLH, dated July, 1997.

Procedure: HMR 3647 was tested in 2 experiments, with or without S9 (prepared from a liver microsomal fraction of rats induced with Aroclor 1254). Dose levels were selected on the basis of pH, solubility and osmolality. Cells (human lymphocytes prepared from whole blood from 2 healthy donors) were exposed to test or control substances, in the presence/absence of S9 (final concentration 2%) for 3 hours at 37'C.

First experiment: Cultures were exposed to test compound or vehicle (DMSO) for 3 hours, then rinsed. Cells were harvested 20 hours after beginning of treatment.
Second experiment: Cultures without S9 were exposed continuously. With S9, cultures were exposed to test or control substances for 3 hours, then rinsed. Cells were harvested 20 and 44 hours after beginning of treatment.

Doses tested:
First experiment:
For treatment: 5.86, 11.718, 23.437, 46.875, 93.75, 187.5, 375, and 750 ug/mL with/without S9.
For chromosome aberration scoring: 93.75, 187.5, and 375 ug/mL without S9
46.875, 93.75, and 187.5 ug/mL with S9

Second experiment:
a. For treatment: 50, 100, 125, 150, 175, 200, and 250 ug/mL without S9
b. For chromosome aberration: 50, 100, 175 ug/mL for 20 hour without S9
100, 200, and 350 ug/mL for 20 hour with S9
250 ug/mL for 44 hour without S9
650 ug/mL for 44 hour with S9

Mitomycin C (MMC) [3 ug/mL for 3 hour treatment, 0.2 ug/mL continuous treatment] and cyclophosphamide (CPA)[50 ug/mL] were used as positive controls for the cells with and without S9, respectively.

Results: Frequency of cells with chromosomal aberrations in vehicle and positive controls were as expected.
Although HMR 3647 was soluble in DMSO at 165 ug/mL a slight precipitate was noted at 750 ug/mL. First experiment: Slight to marked toxicity was observed at doses of >93.75 ug/mL and >23.437 ug/mL, with and without S9, respectively. Doses of 375 and 187.5 ug/mL were chosen as the highest doses for chromosomal aberration scoring.

HMR 3647 did not elicit any significant increases in the frequency of chromosomal aberrations at the doses chosen for cytogenetic analysis. Therefore, the second experiment was conducted.

Second experiment: Slight to moderate toxicity was noted at all dose levels at 20 hours when no S9 was added (-22-87% decrease). When S9 was added, moderate to marked toxicity was noted at all dose levels >200 ug/mL (-54-95% decrease) so 350 ug/mL was chosen as the highest dose for chromosomal aberration scoring, both with/without S9.

HMR 3647 did not elicit any significant increases in the frequency of chromosomal aberrations at the doses selected at any of the harvest times, whether in the absence/presence of S9.

The sponsor concluded that in this study, HMR 3647 did not show any clastogenic potential in cultured human lymphocytes.

Bone Marrow Micronucleus Test by Oral Route in Mice with HMR 3647; Report 13610MAS, dated March, 1997.
Animals: 5 Swiss OF1 mice/sex/dose

Doses administered: 0 (0.5% methylcellulose), 250, 500, or 1000 mg/kg by gavage once, or 50 mg/kg cyclophosphamide (CPA) once (positive control).

CPA induced a significant increase in the frequency of MPE, with the PE/NE ratio being significantly decreased.

In all HMR 3647-treated groups, the frequency of MPE was comparable to respective controls. In the 1000 mg/kg animals, the PE/NE ratio decreased 48 hours after dosing, demonstrating a toxic effect of the test substance.

The sponsor concluded that HMR 3647 did not induce any damage to the chromosomes or the mitotic apparatus in bone marrow cells of mice treated at 250, 500, or 1000 mg/kg orally.

Biological Stability of 3H-RU66647 in the Male and Female Sprague-Dawley Rat; Report #95/8144/CN, dated February, 1996.
Animals: 5 Sprague-Dawley rats/sex/timepoint

Doses administered: 10 mg/kg 3H-RU66647 in an aqueous solution of 75% polyethylene glycol 300. The tritium was labeled on the butyl chain in the beta and gamma positions of the imidazole ring.

Procedure: Blood was collected 6, 24, 48 and 72 hours after treatment.

The sponsor concluded that the detection of radioactivity in the form of tritiated water indicates that the metabolism of the compound affects the position of the label. This indicates that RU66647 undergoes little biotransformation in the rat, but more in the female than the male. Their conclusion that the formation of tritiated water can be considered complete 24 hours after administration in the male and female rat is questionable, as metabolites were not considered.

Study of the Absorption and Absolute Bioavailability of HMR 3647 in the Male Sprague-Dawley Rat After Single Intravenous or Oral Administration of 10 mg/kg of 14C-HMR 3647; Report #96/9019/CN, dated November, 1997. Animals: 5 male Sprague-Dawley rats/dose, aged 7 weeks at study

initiation.

Doses administered: 10 mg/kg by i.v. or oral route

Procedure: Animals were dosed and groups of 5 were killed at the following times: 0.083, 0.25, 0.5, 0.75, 1, 1.5, 2, 3, 4, 5, 6, 8, 24, or 30 hours post-dosing.

Results: After i.v. administration, dispersion between individual concentrations at each sampling time was low: ratio between extreme values varied between 1.2 and 3.1 up to 8 hours post-dosing. The mean plasma concentration after i.v. administration was within 5 minutes of dosing. After oral dosing, the mean maximum concentration was reached 0.25 minutes post-dosing.

After oral dosing, tmax was 0.25 hours. The AUCs (total radioactivity) after both administration routes was 2.52 and 1.24 meq/L/hr for the i.v. and oral routes, respectively. Absorption of HMR 3647 was 47%.

The AUCs (0-infinity) after both administration routes was 2.15 and 0.82 meq/L/hr for the i.v. and oral routes, respectively, and the bioavailability was 36%. Total clearance was 4.36 L/hr/kg, and the t1/2 was 1.7 hours.

The sponsor concluded that after i.v. administration, the volume of distribution was 10x body size, and total clearance was high (0.9 L/hr), and the t1/2 was 1.7 hours.

After oral administration, the maximum plasma concentration was reached 0.25 hours post-dosing, and that after that timepoint, changes in plasma concentrations were irregular. Absorption was 47% and absolute bioavailability (36%) show that a moderate first pass effect occurred.

Tissue Distribution of Radioactivity by Autoradioluminography 2h, 6h, 24h and 72 hours after Oral Administration of A Single Dose of 10 mg/kg of 14C-HMR 3647 in Male Albino Sprague-Dawley Rat; Report #96/8792/CN, dated July, 1997.
Animals: 4 male Sprague-Dawley rats, aged 8 weeks at study initiation.

Doses administered: 10 mg/kg by oral gavage. The 14C-label was on the carbon of the methoxy group in position 6 of the erythromycin.

Procedure: Animals were dosed, then sacrificed at 2, 6, 24, and 72 hours post-dosing. In an exposure cassette, sections were placed in direct contact with imaging plates and exposed for 72 hours.

The sponsor concluded that:

1) Substantial but relatively low levels at 2 and 6 hours were found in all tissues, but the CNS was below level of quantitation.

2) Tissue concentrations in most tissues were comparable at 2 and 6 hours.

3) HMR 3647 has a large volume of distribution, evidenced by low blood levels

4) Almost total elimination of radioactivity by 24 hours post-dosing

5) Persistence of radioactivity in GI tract contents for 72 hours

6) Elimination primarily in bile and to a lesser extent in urine

Cytochrome P450 Isoenzymes Involved in HMR 3647 Metabolism by Rat Liver Microsomes; Report #97/9953/CN, dated November, 1997.
Animals: 3 rats/group for HMR 3647-treated microsomes with inhibitors; 1/group for control microsomes. Biological material was liver from control and pre-treated rats with phenobarbital/3 methylcholanthrene or dexamethasone.

Qualitative analysis: Metabolic profiles showed nine chromatographic peaks, excluding HMR 3647 and its alpha epimer. Two of the peaks were identified as N-oxide pyridine, and N-desmethyl desosamine, respectively. These two metabolites represent >50% of the total metabolites produced.

HMR 3647 was biotransformed by rat liver microsomes. For liver microsomes from rats treated by dexamethasone, piperonyl butoxide inhibition was about 86%, while for controls, it was ~50%. For microsomes from rats pre-treated with phenobarbital and/or 3- methylcholanthrene, the level of inhibition was ~0%.
The level of biotransformation in the presence of degradation product was higher compared to the level of biotransformation with re-purified HMR 3647 with control, phenobarbital and dexamethasone microsomes. These results seem to indicate that the degradation product was not transformed into detectable metabolites.

Results indicated that the biotransformation of HMR 3647 and metabolite formation were under the control of CYP3A family of isozymes.

The sponsor concluded that HMR 3647 was mainly metabolized in rat liver microsomes by CYP3A1/3A2. The rate of metabolism by liver microsomes from rats pretreated with dexamethasone (CYP3A induction) was 0.71 nmol/min per nmol of CYP and HMR3647 had a 'mild' affinity for Phase 1 enzymes.

CONCLUSIONS:
The safety pharmacology study data indicate that HMR 3647 has a significant adverse effect on the gastrointestinal tracts of dogs (vomition), and rats (delayed gastric emptying).

The sponsor concluded that 50 mg/kg/d was the NOEL for the 4 week oral toxicity study in rats on the basis of cecal enlargement, hepatoxicity at the mid and high doses, and phospholipidosis (a known class effect of macrolides) at the 150 and 300 mg/kg/d doses.

The sponsor considers 90 mg/kg/d to be the NOEL for direct systemic toxicity for this four week intravenous study in rats. However, the hematologic and clinical chemistry findings are significant in that no significant histopathologic lesions were noted to support the claim that the inflammatory syndrome was due to the method of administration. Therefore, the NOEL for this study is determined to be 10 mg/kg/d.

Canine heart rates were significantly increased (1 male, 2 females) at doses of 300 mg/kg given orally. The sponsor considered the elevated rates to be a stress response and questioned the relationship to treatment.

Ptyalism was noted in all mid and high dose animals. Small head and chewing movements were observed in one low dose female, 2 mid dose males, and all mid dose females, and high dose animals of both sexes. Gastrointestinal hypomotility was noted in one mid dose male and all mid dose females, and all high dose animals, with tremors and/or hypotonia. Dehydration was recorded in all of these animals. Significant changes in ALT and AST were found in the mid and high dose animals at both timepoints.

Histiocytosis (foamy macrophage infiltration) was noted in most of the high dose animals, and some of the mid dose animals. These changes are related to a phospholipidosis, previously described with the compound.
The sponsor concluded that the NOEL for the 30 day oral toxicity study in dogs was 50 mg/kg/d based on the vomition, body weight decreases, nephrotoxicity, hepatotoxicity and the poor clinical condition.

The sponsor concluded that HMR 3647, daily for 30 days by the intravenous route, was clinically well tolerated at 10 and 30 mg/kg/d, but elicited vomiting and ptyalism in animals given 90 mg/kg/d. They set the NOAEL at 90 mg/kg/d.

Fertility study: The sponsor concluded that HMR 3647 was well tolerated at 50 and 150 mg/kg/d, but poorly tolerated at 300 mg/kg/d in the rat. Male and female fertility indices were reduced at 150 and 300 mg/kg/d. Their proposed NOEL was 150 mg/kg/d for parental tolerance, 50 mg/kg/d for gametes and fertilization, and 300 mg/kg/d for mating behavior and pre-implantation/implantation. However, the overall NOEL for the study is set at 50 mg/kg/d due to the reductions in fertility indices and fertilization.

In the rat embryo-fetal development study, the NOEL for maternal tolerance was 50 mg/kg/d, and for embryo-fetal development was 150 mg/kg/d.

In the rabbit embryo-fetal development study, the NOEL for effects on maternal and embryofetal toxicity was 20 mg/kg/d, and the NOAEL was 60 mg/kg/d.

Genetic Toxicology:
HRM 3647 induced toxicity at all doses >1 ug/plate for the Salmonella strains, and >10 ug/plate for the E. coli strain. It did not induce any significant increases in revertants, whether in the presence/absence of S9.

The sponsor concluded that HMR 3647 did not show any mutagenic activity in this assay with S. typhimurium or E. coli.

In the mouse lymphoma assay, in the absence of S9, moderate to very marked cytotoxicity was observed at doses >31.25 ug/mL (relative cloning efficiency between 74% - 3% depending on dose). At lower doses, no appreciable cytotoxicity was found.

In the presence of S9, cytotoxicity was less marked (relative cloning efficiency decreased between 26% and 94% depending at doses >125 ug/mL).

The sponsor concluded that HMR 3647 did not show any mutagenic activity in the mouse lymphoma assay.

HMR 3647 did not elicit any significant increases in the frequency of chromosomal aberrations at the doses selected at any of the harvest times, whether in the absence/presence of S9. The sponsor concluded that in this study, HMR 3647 did not show any clastogenic potential in cultured human lymphocytes.

RECOMMENDATION:

Taking into consideration the NOEL in the rat i.v. study (10 mg/kg/d providing 0.12x the proposed human dose on a mg/m2 basis), the rat oral toxicity study (50 mg/kg/d providing 1.6x the proposed human dose on a mg/m2 basis), the dog i.v. study (90 mg/kg/d providing 3.7x the proposed human dose on a mg/m2 basis), and the dog oral toxicity study (50 mg/kg/d providing 2.03x the proposed human dose on a mg/m2 basis), the results vary significantly with respect to species and route of administration. Therefore, the results of the comparative metabolic profile studies, including the human plasma levels, should be integrated into the assessment of the potential human health risk from compound administration.

The review above should be considered a portion of the NDA review for this ketolide and it is fileable.

GS

HFD-520: Orig. IND, NDA
HFD-520/PT/Peters
HFD-520/MO/Davidson
HFD-520/Chem/Yu
HFD-520/CSO/Cintron
Draft date (# of drafts): #1; 3/13/00

Concurrence Only:
HFD-520/DepDivDir/Gavrilovich
HFD-520/PTTeamLdr/Osterberg

GS
GS

Review and Evaluation of Pharmacology and Toxicology Data
Division of Anti-Infective Drug Products, HFD-520

NDA#: 21144.AM

Date CDER Received/Type of Submission: 2/27/01
Reviewer: Terry S. Peters, D.V.M.
Date Assigned: 3/1/01
Number of Volumes: 2
Date Review Started: 3/5/01
Date 1ST Draft Completed: 3/7/01
Scientific Literature Reviewed: No

KEY WORDS: Ketek, telithromycin, cardiovascular dog study, toxicology study

Sponsor: Aventis Pharmaceuticals, Kansas City, MO
Contact person:
Manufacturer for drug substance: Hoechst Marion Roussel, Cedex, France

Drug:
Code Name: HMR3647
Generic Name: telithromycin
Trade Name: Ketek® (proposed)
Chemical Name:11,12-dideoxy-3-de[(2,6-dideoxy-3-C-methyl-3-O-methyl-alpha-L-ribo-hexo-pyranosyl)oxy]-6-O-methyl-3-oxo-12,11-[oxycarbonyl[[4-4-(3-pyridinyl)-1H-imidazol-l-yl)butyl]imino]]-erythromycin

Drug Class: Ketolide

Indication: Semisynthetic ketolide (macrolide) with reported efficacy against Gram positive bacteria, especially those resistant to erythromycin A. The mechanism of action is speculated to be by binding to two different sites on the ribosome, blocking the production of methylase. The sponsor is requesting approval for the following indications: 1) Community acquired pneumonia due to *S. pneumoniae*, including strains resistant to penicillin and erythromycin, *H. influenzae*, *H. parainfluenzae*, *M. catarrhalis*, *C. pneumoniae*, *L. pneumophila*, and/or *M. pneumoniae*,
2) Acute sinusitis due to *S. pneumoniae*, including strains resistant to penicillin and erythromycin, *H. influenzae*, *H. parainfluenzae*, *M. catarrhalis* and/or *S. aureus*
3) Tonsillitis/pharyngitis due to *S. pyogenes*

Proposed Clinical Use: 800 mg/day qd administered as a 400 mg tablet for 7-10 days in CAP and 5 days for other indications.

Route of Administration: Oral

Cardiovascular effects: Effect of a 15-minute intravenous infusion of HMR 3647on electrocardiogram and hemodynamics in conscious dogs with plasma concentration determination/Comparison with clarithromycin; R2000PHM0414. This study was performed at Aventis Pharma, Cedex, France and was initiated on 7/21/00. The protocol for this study was not submitted to the FDA for review prior to study initiation.

The purpose of this study was to determine the effect of HMR 3647 in comparison to clarithromycin with respect to heart rate, EKG tracings and the PK/PD relationships (if any). A wash-out period of 48 hours was given between dosings. Dogs were implanted with a telemetry device and an external jugular catheter at least 2 weeks before study initiation.

HMR 3647 was "98.8% of HMR 3647 + RU73031 (2α-epimer) as is".

Clarithromycin lactobionate was the comparator (Klacid® I.V. [Abbott]), lot #37688T823. As this product is not approved in the United States, minimal information is available as to the biological effect of this salt in vivo.

The initial cardiovascular parameters were obtained as the average of 3 periods of 30 seconds at about 10 minute intervals during the hour pre-dosing. During the infusion, arterial blood pressure, heart rate and EKG parameters were calculated as an average of 30 seconds of recording at 5, 10 and 15 minutes post-initiation and at 2, 5, 10, 15, 20, 30, 45, and 60 minutes after the end of the infusion. The following statement appears in the submission: "Sometimes, portions of tracings close to the above mentioned times were chosen as more representative values." Blood samples were taken at the same time points except in controls where samples were only taken before the infusion.
First part of the study: HMR 3647 was administered over 15 minutes at 2.4, 6 or 15 mg/kg to 2 male and 6 female beagles. "Eight conscious dogs received in turn either one dose of HMR 3647 or saline." The results were compared to saline. Plasma concentrations were obtained.

Second part of the study: Clarithromycin was administered over 15 minutes at 2.4, 6 or 15 mg/kg to the same animals. Plasma concentrations were obtained.

Results: Plasma concentrations of HMR 3647 were assayed at Aventis Pharma, Romainville, France using HPLC and fluorescence detection. Samples for clarithromycin were assayed at [redacted] using liquid chromatography [redacted] mass spectrometry.

Ketek portion of the study:
In the data evaluation section the following statement is found: "Due to missing values for blood pressure for some time points, dog 1162 was not included in data analyses for this parameter." This is an unusual procedure for a GLP study as all data should be included in the analyses.

Neither drug had a statistically significant effect on blood pressure, PQ intervals or QRS complexes. Statistical analyses did not reveal differences between vehicle and drug treatment. However, HMR 3647 elicited a QT prolongation at 15 mg/kg using Bazett's. An increase in corrected QT of 10-15 ms was observed with saline, 2.4 or 6 mg/kg while an increase of 25 ms (considered a moderate lengthening of QT) was noted at the end of the infusion period at 15 mg/kg. When using Fridericia's correction, the increases were 4 ms at the end of the 6 mg/kg infusion and 11 ms at 15 mg/kg at the end of the infusion.

HMR 3647 elicited an increase in heart rate regardless of treatment (maximal 53 bpm for saline, 2.4 [maximal 49 bpm] or 6 mg/kg [maximal 48 bpm] groups) but especially at 15 mg/kg (up to 55 bpm) peaking ~5 minutes after the end of the infusion). Bpm are actual differences from initial values.

PK analysis showed a biologically significant increase in heart rate and corrected QT intervals at the highest doses. However, the coefficients of variation were large (40-58%) with HMR 3647. Cmax concentrations were seen at 5, 10 or 15 minutes sampling times. Mean Cmaxs for Ketek were 3.76± 0.55, 7.6± 1.1 and 21.9± 2.9 mg/L at 2.4, 6 and 15 mg/kg, respectively. Thus, the increases were close to dose proportional.

Clarithromycin portion of the study: Emesis was reported in 1 animal at 6 mg/kg and 4 animals at 15 mg/kg.

Statistical analysis of QT interval showed a "tendency" to be shortened (p= 0.096). This effect was noted from the end of the infusion for up to 10 minutes.
An increase in heart rate (maximal 53 bpm) was noted in the saline treated animals. A significant and dose-dependent increase in heart rate was noted with clarithromycin (p=0.0039) at 6 (maximal 69 bpm) and 15 mg/kg (Maximal 88 bpm). Once correction for Bazett's formula was done, the 6 mg/kg dose increase (+23 ms) was statistically significant as was the 15 mg/kg dose (+24 ms). Levels slowly returned to baseline by the end of the observation period. No significant increases were found using Fridericia's formula.

PK analysis showed a biologically significant increase in heart rate and Bazett's corrected QT intervals. However, the coefficients of variation were large (41-68%) with clarithromycin. Cmax concentrations were seen at 5, 10 or 15 minutes sampling times. Mean Cmaxs for clarithromycin were 8.57± 0.85, 12.2± 1.5 and 21.5± 3.3 mg/L at 2.4, 6 and 15 mg/kg, respectively. Cmax and AUC (0-75 minutes) increased with dose with less than proportionality with respect to dose.

PK/PD relationship: Changes in QTc were analyzed versus the mean maximum plasma concentrations. No biologically significant increase was reported at 2.4 mg/kg with mean Cmax of 8.6 mg/L. Significant increases were found at 6 and 15 mg/kg with mean Cmaxs of 12.2 and 21.5 mg/L of clarithromycin in plasma, respectively.

In previous studies, the sponsor concluded that the threshold for these changes was ~5 mg/L of unbound drug. In this study, the sponsor concludes that "clarithromycin shows the same properties as HMR 3647 on heart rate and corrected QT... The threshold concentration of clarithromycin is probably very close to that of HMR 3647 or even slightly lower since a biologically relevant effect on both parameters was observed from the dose of 6 mg/kg which gave peak plasma concentrations of about 12 mg/L (about 4 mg/L of unbound drug)."

OVERALL SUMMARY AND EVALUATION:

Introduction: The purported purpose of this study was to compare the cardiovascular effects of HMR 3647 and clarithromycin given intravenously at 0, 2.4, 6 or 15 mg/kg as a single dose.

Safety Evaluation: Although the results appear comparable, the differences between the drugs with respect to t ½, dose proportionality, and Cmax/AUC make the comparison between the compounds less than meaningful. Additionally, in previously reviewed studies, the maximal cardiovascular effects were reached 4-6 hours post-dosing and these timepoints were not evaluated in this study.

Conclusions: This study does not add significant information for the cardiovascular effects of telithromycin.

RECOMMENDATIONS: NAI

/S/

Terry S. Peters, D.V.M.
Veterinary Medical Officer, HFD-520

Orig. NDA 21144
cc:
HFD-520
HFD-520/Pharm/Peters
HFD-520/MO/Davidson, Ross, Korvick
HFD-520/Chem/Yu
HFD-520/CSO/Cintron
HFD-520/Micro/Marsik
HFD-520/BioPharm/Zheng

Concurrence Only:
HFD-520/REOsterberg
HFD-520/LGavrilovich /S/