CENTER FOR DRUG EVALUATION AND RESEARCH

APPLICATION NUMBER:

21-144

PHARMACOLOGY REVIEW(S)

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

NDA #: 21144, BP

Date CDER Received/Type of Submission: 1/17/01; BP- additional studies
Reviewer: Terry S. Peters, D.V.M.
Date Assigned: 1/17/01
Number of Volumes: 1 CD
Date Review Started: 1/19/01
Date 1ST Draft Completed: 2/21/01
Scientific Literature Reviewed: Yes

KEY WORDS: Ketek, animal toxicology studies, genetic toxicology, ADME

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 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.

Review Contains Information to be Communicated to Sponsor: No

Studies reviewed within this submission:

1) In Vitro Mammalian Chromosome Aberration Test with RU71094 in Cultured Human Lymphocytes, Report #19829
2) In Vitro Mammalian Cell Gene Mutation Test with RU71094 in L51784 TK± Mouse Lymphoma Cells; Report #19830
3) RU76363: Mutation Assay on Mouse Lymphoma L5178Y Cells At The Thymidine Kinase Locus; Report #RPR/RD/SA/CRVA 00-338
4) In Vitro Mammalian Chromosome Aberration Test with RU76363, in Cultured Human Lymphocytes; Report #19831
5) Metabolic Profiles of HMR 3647 in Rabbit Plasma; Report #R2000KIN0401
6) In Vitro Effect of HMR 3647 and Its Metabolites RU 71094 and RU 76363 on the Contractions of Guinea Pig Ileum Induced by Field Stimulation; Report #R2000PHM0158

7) **Study of RU71094, RU76363 and HMR 3647 in the Choline Uptake Binding and Acetylcholinesterase Assays; Report #R2000PHM0132**
8) **Study of RU71094, RU76363 and HMR 3647 on Phosphodiesterase VI Activity; Report R2000PHM0358**
9) **Re-Evaluation of Eye Sections from a 28-Day Oral (Gavage) Administration Sub-Chronic Toxicity Study of HMR 3647 in Cynomolgus Monkeys; Report CLE 552-037**

SAFETY PHARMACOLOGY

Gastrointestinal effects: **In Vitro Effect of HMR 3647 and Its Metabolites RU 71094 and 76363 on the Contractions of Guinea Pig Ileum Induced by Field Stimulation; Report #R2000PHM0158.** In this study, the effect of the ketolide and its major metabolites on electrically induced contractions of isolated male guinea pig ileum (N=8) at concentrations of 10-6, 3x 10-6, 10-5, 3x 10-5 and 10-4 M was evaluated. HMR 3647 induced slight (albeit significant) decrease at 10-4 and 10-5 M when compared to the baseline value. The metabolites also showed a decrease (slightly significant). The positive control (metoclopramide) increased the contractile force significantly (45%). The sponsor concluded that: "HMR 3647 as well as its main metabolites do not seem to induce any increase in acetylcholine release."

PHARMACOKINETICS/TOXICOKINETICS

Metabolism: **Metabolic Profiles of HMR 3647 in Rabbit Plasma; Report #R2000KIN0401.** The purpose of this study was to compare the metabolic profiles in male and female New Zealand White rabbits as well as mated Himalayan rabbits. The Himalayans were sampled at 4 hours post-dosing of 60 or 180 mg/kg on Days 6 and 18 post-mating, and male New Zealand White rabbits at 4 hours post-dosing of 60 mg/kg on Days 6 and 18 post-mating, and female New Zealand White rabbits at 3 hours of 100 mg/kg single dose.

Metabolite identification was performed by LC/MS/MS as well as [redacted]. Quantification was achieved by [redacted] analysis of selected plasma samples.

Results: The parent compound was consistently the major drug component. The main metabolite (3-27% of Himalayan plasma, 2-9% of the New Zealand plasma) was the N-desmethyl-desosamine. The other metabolites were M4'b (the alcohol from the loss of aryl rings) [1-13% in the Himalayan rabbit, <1% in the New Zealand White rabbit] and N-oxide pyridine (1-6% in both types of rabbit). Inter-animal variability was high. Although the metabolic profiles were qualitatively similar, the quantitative differences between rabbit strains are not explained. From the submission: "Whatever the strain, following oral administration of 60 mg/kg to mated females, a concomitant decrease in the plasma concentrations of HMR 3647 and all of its metabolites, ..., occurred between Day 6 and 18 post-coitum."

Study of RU71094, RU 76363, and HMR 3647 in the Choline Uptake Binding and Acetylcholinesterase Assays; Report R2000PHM0132. The purpose of this study was to assess the affinity of the various test compounds (RU71094- pyridino imidazole; RU76363- a metabolite of HMR 3647; HMR 3647) to the choline uptake site in a binding assay and effects on the electric eel acetylcholinesterase activity. Each of the compounds was tested at 1, 3, 10, and 30 μM in duplicate samples.

Results: Each of the compounds elicited inhibition (<30%) at the highest concentration tested in the choline uptake assay. No acetylcholinesterase inhibition was noted with any compound.

Study of RU 71094, RU 76363 and HMR 3647 on Phosphodiesterase VI Activity; Report R2000PHM0358. This study was conducted to determine the in vitro effects of the various compounds on phosphodiesterase VI activity. Compounds were tested at 1, 10 and 100 μM in duplicate samples.

Results: HMR 3647 elicited a 40% inhibition at 100 μM but did not have any effect at the lower concentrations. The other test compounds did not elicit any inhibition at any dose.

Re-Evaluation of Eye Sections from a 28-Day Oral (Gavage) Administration Sub-Chronic Toxicity Study of HMR 3647 in Cynomolgus Monkeys; Study #CLE 552-037. This study was previously

reviewed but this report is from the peer review performed at the request of the sponsor. As in the previous report, no significant ocular histopathologic findings were associated with treatment with HMR 3647.

Study of the In Vivo β-Oxidation of Fatty Acids After a Single Intraperitoneal Administration of HMR 3647 in Rats; Report #R2000TOX0012. The purpose of this study was to assess the potential inhibition of mitochondrial β-oxidation after administration of 150 mg/kg i.p. HMR 3647 as compared to clarithromycin and azithromycin at the same dose.

The animals were treated with test compound, then 15 minutes later, were administered ^{14}C-palmitic acid by gavage. For 4 hours, the animals were placed in a glass box with ^{14}C-CO2 measured in the outflow. Samples were evaluated at 30, 60, 120, 180 and 240 minutes.

Results: The sponsor used 180 minutes as the most representative time "from the literature". At that time, decreased CO2 was 39%, 38% and 33% for HMR 3647, clarithromycin and azithromycin, respectively. However, a very different outcome was found at 60 minutes- the % exhaled CO2 was –67%, +13% and –63% for the respective compounds. Only 1 male animal was tested for the macrolides, but 4 were tested for the control database. It is difficult to evaluate the usefulness of these data based upon the small numbers tested.

GENETIC TOXICOLOGY

Study Title: **In Vitro Mammalian Chromosome Aberration Test with RU71094 in Cultured Human Lymphocytes,**
Study No: **Report #19829**
Volume # and Page #: CD only
Conducting Laboratory: [redacted]
Date of Study Initiation/completion: 3/29/00
GLP Compliance: Yes
QA- Reports Yes
Drug Lot Number: 8D1006C9
Study Endpoint: Drug-induced chromosomal aberrations in cultured human lymphocytes

Methodology:
- Strains/Species/Cell line: Cultured human heparinized whole blood (48 hour incubation with PHA)
- Dose Selection Criteria:
  - Basis of dose selection: pH, osmolality and solubility
  - Range finding studies: None
- Test Agent Stability:
- Metabolic Activation System: S9 from rat livers induced with Aroclor 1254
- Controls:
  - Vehicle: DMSO
  - Negative Controls: None
  - Positive Controls: Cyclophosphamide (with S9) and mitomycin C (without S9)
  - Comments:
- Exposure Conditions:
  - Incubation and sampling times: First experiment: Cells were incubated for 3 hours, rinsed and harvested 20 hours after beginning of treatment. In the second experiment, cells were incubated continuously (without S9) or for 3 hours (S9-treated). Cells were harvested 20 or 44 hours post-initiation of treatment.
  - Doses used in definitive study: First experiment: 0.08, 0.16, 0.31, 0.63, 1.25, 2.5, 5 and 10 mM. Second experiment: 0.63, 1.25, 2.5, 5, 7.5 and 10 mM
- Analysis: Performed by [redacted]
  - No. slides/plates/replicates/animals analyzed: 200 metaphases/dose level.
  - Counting method: Microscopic

- Cytotoxic endpoints: Mitotic index on a total of 1000 cells
- Genetic toxicity endpoints/results: Metaphase analysis with structural aberrations and numerical aberrrations for 2.5-10 mM concentrations for the 3 hour exposure, 1.25-5 mM concentrations for the 20 hour exposure, and 2.5 mM concentration for the 44 hour exposure.
- Statistical methods:

Results:

- Study Validity: Study is considered valid as the frequency of structural aberrations in vehicle and positive controls were as specified in the acceptance criteria
- Study Outcome: Without S9: First experiment: A significant decrease in mitotic index was appreciated at doses ≥5 mM. Second experiment: Toxicity was noted after 20 hours of exposure at ≥2.5 mM (significant decrease in mitotic index). After 44 hours of exposure, notable toxicity was appreciated at ≥1.25 mM.

With S9: First experiment: A marked decrease in mitotic index at ≥0.31 mM (no clear dose relationship). Second experiment: At 20 hours, a 46% decrease in mitotic index (no clear dose relationship). At 44 hours, a slight (36%) decrease in mitotic index was reported.

Summary: No significant increases in frequency of cells with structural chromosomal aberrations in either experiment. No clastogenic potential was shown in this experiment with RU71094.

Study Title: **In Vitro Mammalian Cell Gene Mutation Test with RU71094 in L5178Y TK$^{\pm}$ Mouse Lymphoma Cells**

Study No: R2000TOX0106, also 19830 MLY

Volume # and Page #: CD only

Conducting Laboratory: [redacted]

Date of Study Initiation/completion: 3/31/00

GLP Compliance: Yes

QA- Reports Yes

Drug Lot Number: 8D1006C9 of RU 71094 (a metabolite of HMR 3647)

Study Endpoint: Evaluation of the potential of RU 71904 to induce mutations at the thymidine kinase

Methodology:

- Strains/Species/Cell line: L5178Y mouse lymphoma cells
- Dose Selection Criteria:
  - Basis of dose selection: Preliminary cytotoxicity test at 0.31, 0.63, 1.25, 2.5, 5 and 10 mM of RU 71094
- Test Agent Stability: Freely soluble in vehicle
- Metabolic Activation System: S9 fraction from rat livers induced with Aroclor 1254
- Controls:
  - Vehicle: DMSO
  - Positive Controls: Methylmethane sulfonate (without S9) and cyclophosphamide (with S9)
- Exposure Conditions:
  - Incubation and sampling times: 0.5x 10^6 cells (3 hour treatment) or 1.5x 10^6 cells (24 hour treatment) with or without S9.
  - Doses used in definitive study: First experiment: 0.31, 0.63, 1.25, 2.5, 5 and 10 mM. Second experiment: 0.63, 1.25, 2.5, 5 and 10 mM (with S9) and 0.31, 0.63, 1.25, 1.88, 2.5, 3.75, and 5 mM (without S9)
- Analysis:
  - No. slides/plates/replicates/animals analyzed: Two plates/dose level with 2000 cells/well to select the mutant cells. For scoring, a well with mutant colony, and a well with no mutant colony were analyzed using a [redacted] and manual counting.
  - Statistical methods: None were necessary
- Criteria for Positive Results: Two fold increase in mutant frequency when compared to the vehicle controls and/or a dose response.

Results:

- Study Validity: In the first experiment (with S9), the vehicle control mutation frequency was > the acceptance criteria ($2.64x\ 10^{-6}$ vs. $60\text{-}250x\ 10^{-6}$). Since the positive controls were clearly positive (5.2x increase), the study was considered valid.

- Study Outcome: First experiment: After the 3 hour incubation without S9, neither cytotoxicity nor mutation frequency changes were appreciated at any dose.

In the 24 hour incubation, cytotoxicity was considered severe (data not shown).

Second experiment: Without S9: At ≥3.75 mM, cytotoxicity was severe so the analysis was only performed on the 0.31- 2.5 mM dose cultures. No biologically significant changes in mutation frequency were appreciated at any dose. A slight statistical significance was found using regression analysis of the mutation frequency.
With S9: A slight cytotoxicity was found but no change in mutation frequency was reported.

Summary: The metabolite (RU71094) did not show mutagenic potential in this test system.

Study Title: **RU76363: Mutation Assay on Mouse Lymphoma L5178Y Cells at the Thymidine Kinase Locus**
Study No: SC/CRVA 00-338
Volume # and Page #: CD only
Conducting Laboratory: Aventis Pharma Recherche-Developpement, Alfortville, France
Date of Study Initiation/completion: 7/18/00
GLP Compliance: Yes (OECD)
QA- Reports: Yes
Drug Lot Number: Batch #3
Study Endpoint:

Methodology:

- Strains/Species/Cell line: L5178Y mouse lymphoma cells
- Dose Selection Criteria:
  - Range finding studies: (Data not provided) Doses used were 100, 200, 375, 750, 1500 and 3000 µg/mL. Excessive cytotoxicity was reported so another assay was performed with maximum doses of 1300 µg/mL without S9 and 1400 µg/mL with S9.
- Test Agent Stability: Stable in DMSO
- Metabolic Activation System: S9 from rat livers induced with Aroclor 1259
- Controls:
  - Vehicle: DMSO
  - Positive Controls: Methylmethanesulfonate (without S9) and benzo(a)pyrene (with S9)
- Exposure Conditions:
  - Incubation and sampling times: First assay: With/without S9: 3 hours of incubation. Second assay: 24 hours without S9, 3 hours with S9.
  - Doses used in definitive study: First experiment: 200, 300, 400, 500, 600, 700, 800, 900, 1000, 1100, 1200 and 1300 µg/mL without S9; 300, 400, 600, 800, 900, 1000, 1100 and 1200 µg/mL with S9.
  - Study design: Cells were exposed to test compound for 3 hours and 24 hours without S9 and 3 hours with S9. Cultures were incubated for 48 hours, then cloned and incubated for 10-13 days with a selection agent (trifluorothymidine).
- Analysis:
  - No. slides/plates/replicates/animals analyzed: Two/dose
  - Counting method: Visual examination as well as large and small colony evaluation
  - Cytotoxic endpoints: Survival
  - Genetic toxicity endpoints/results: Mutant cell population
- Criteria for Positive Results: All of the following criteria had to be met:

1) Statistically significant increase in the corrected mutant frequency for ≥1 dose
2) Increase is concentration-related or observed at the highest concentration

3) Increase is reproducible
4) Corrected mutant frequency is out of the range of the negative historical controls

Results:

- Study Validity: Vehicle and positive control cultures performed as expected.
- Study Outcome: First experiment- 3 hour cultures (without S9): Marked cytotoxicity was reported at ≥600 µg/mL. No statistically significant increases in mutant frequency were noted in any evaluable culture.

Three hour cultures (with S9): Marked cytotoxicity was reported at ≥1200 µg/mL. No statistically significant increases in mutant frequency were noted in any evaluable culture.

Second experiment- 24 hour cultures (without S9): Marked cytotoxicity was noted at ≥250 µg/mL. No statistically significant increases in mutant frequency were noted in any evaluable culture. Three hour cultures (with S9): No statistically significant increases in mutant frequency were noted in any evaluable culture.

Summary: The test compound is considered negative in this mouse lymphoma (L5178Y cells) assay under the conditions of this experiment.

Study Title: **In Vitro Mammalian Chromosome Aberration Test with RU76363 in Cultured Human Lymphocytes**
Study No: R2000TOX0105, also MLH 19831
Volume # and Page #: CD only
Conducting Laboratory: [redacted] Analysis of the slides was performed by [redacted]
Date of Study Initiation/completion: 7/12/00
GLP Compliance: Yes
QA- Reports Yes
Drug Lot Number: RU76363 is a metabolite of HMR 3647
Study Endpoint: Induction of chromosomal aberrations in cultured human lymphocytes

Methodology:

- Strains/Species/Cell line: Human lymphocytes from 2 healthy donors
- Dose Selection Criteria:
  Previous work with the compound (not presented)
- Test Agent Stability: Stable in the vehicle
- Metabolic Activation System: S9 from rat livers induced with Aroclor 1259
- Controls:
  - Vehicle: DMSO
  - Positive Controls: Mitomycin C (without S9) and cyclophosphamide (with S9)
- Exposure Conditions:
- Incubation and sampling times: First experiment: Cultures were exposed to test/control compound, with/without S9 for 3 hours, then rinsed. They were treated with colcemid and harvested 20 hours after initiation.

Second experiment: Without S9: Cells were exposed continuously; with S9: cells were exposed for 3 hours, then rinsed. Harvests were 20 or 44 hours post-initiation.

- Doses used in definitive study: First experiment : 46.88, 93.75, 187.5, 375, 750, 1500, 2250, and 3000 µg/mL (with/without S9)

Second experiment: Without S9: 62.5, 125, 250, 375, 500, 750, 1000 and 1500µg/mL; with S9: 125, 250, 500, 750, 1000, and 1500 µg/mL

- Analysis:
  - No. slides/plates/replicates/animals analyzed:
  - Counting method:
  - Cytotoxic endpoints: Mitotic index for 1000 cells
  - Genetic toxicity endpoints/results: Metaphase evaluation for 3 dose levels and controls from 200 cells (100/culture when possible)

- Criteria for Positive Results: "A reproducible and statistically significant increase in the frequency of cells with structural chromosome aberrations for at least one of the dose-levels and one of the two harvest times."

Results:

- Study Validity: Positive and vehicle controls performed as expected.
- Study Outcome: First experiment: Without S9: A significant decrease in mitotic index was reported at all doses ≥750 µg/mL. Only cells from the female donor were scored for metaphases.

With S9: A decrease in the mitotic index was noted at ≥375 µg/mL.
Second experiment: Without S9: After 44 hours of exposure, significant toxicity was reported at ≥750 µg/mL.
With S9: A significant decrease in mitotic index was reported for the 20 hour harvest, and a modest decrease for the highest dose in the 44 hour harvest.

Summary: No statistically significant increases in structural chromosomal aberrations were reported in the first experiment. In the second experiment, structural chromosomal aberrations reached 2.5% in the 1000 µg/mL cultures. No increase was noted in the first experiment but the sponsor considered this finding to not be biologically relevant as it wasn't statistically significant and was within historical control ranges (0-3%).

RECOMMENDATIONS:
No action is indicated at this time.

LS I

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

Orig. NDA
cc:
HFD-520/Davidson
HFD-520/Pharm Team Ldr/Osterberg
HFD-520/Pharm/Peters
HFD-520/Chem/Yu
HFD-520/CSO/Cintron
HFD-520/Micro/Marsik

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

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SEP 7 2000

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: 9/6/00
IND/NDA number: NDA 21-144
Serial number/date/type of submission: 7/3/00
Information to sponsor: Yes
Sponsor (or agent): 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 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.

Studies reviewed:
1) Electrophysiological Effects of the Concomitant Application of Sotalol and Clarithromycin on Isolated Rabbit Purkinje Fibers; 99/11310/PH
2) Electrophysiological Effects of the Concomitant Application of the Anti-Arrhythmic Quinidine with Clarithromycin on Isolated Rabbit Purkinje Fibers; 99/11292/PH
3) Plasma Profiles with HMR 3647 and Clarithromycin in Rat Models with Hepatic Injury Induced by Carbon Tetrachloride and 1-Naphthylisothiocyanate; LPK-98-012
4) Single Dose Toxicity Study of HMR 3647 Administered Orally to Beagle Dogs; Study SBL-7891
5) Antigenicity Study of HMR 3647; SBL 78-92

SAFETY PHARMACOLOGY:

Cardiovascular effects:
1) **Electrophysiological Effects of the Concomitant Application of Sotalol and Clarithromycin on Isolated Rabbit Purkinje Fibers; 99/11310/PH.** This study was conducted by HMR, Cedex, France, and was initiated on 7/23/99.

The purpose of this study was to determine the in vitro effects of sotalol (anti-arryhthmic compound) with clarithromycin on the electrical activity of isolated male rabbit Purkinje fibers.

Sotalol concentration: 5 μM
Clarithromycin concentrations: 3, 10 and 30 μM

Results: Clarithromycin produced a concentration dependent lengthening of the action potential duration (40 ms, 78 ms, and 130 ms for the respective doses). The percent APD90 increases were 15, 46, and 82 at 3Hz, 1Hz and 0.2Hz, respectively. No early repolarizations were triggered at the low rate. The lengthening of the action potential can be explained by inhibition of the Ikr (rapid component of the delayed rectifier potassium channel) current.

Sotalol produced an increase in action potential duration (86 ms).

Neither compound elicited changes in the resting membrane potential, action potential amplitude nor Vmax.

When the compounds were applied concomitantly, an additive effect was noted in the action potential lengthening. Early after depolarizations were reported at the low rate (1 fiber of 6 for sotalol + clarithromycin at 3 μM).

The sponsor concluded that the coadministration of clarithromycin with anti-arrhythmic drugs having Class III activity should be considered with caution.

2) **Electrophysiological Effects of the Concomitant Application of the Anti-Arrhythmic Quinidine with Clarithromycin on Isolated Rabbit Purkinje Fibers; 99/11292/PH.** This study was conducted by HMR, Cedex, France, and was initiated on 7/23/99.

The purpose of this study was to determine the in vitro effects of quinidine (anti-arryhthmic compound) with clarithromycin on the electrical activity of isolated male rabbit Purkinje fibers.

Quinidine concentration: 1 μM
Clarithromycin concentrations: 3, 10, and 30 μM

Results:
Quinidine elicited produced an increase of action potential duration and a depression of the Vmax.

Clarithromycin results are reported for the study above but the percent increases were 15, 46 and 68, respectively.

When the test compounds were administered concomitantly, the effects were additive on action potential duration (basal and high stimulation rates). At the low stimulation rate (1 pulse/2 sec), the action potential duration prolongation was potentiated by co-administration.

Neither compound elicited changes in the resting membrane potential, nor action potential amplitude.

The sponsor concluded that the coadministration of clarithromycin with anti-arrhythmic drugs having Class III activity should be considered with caution.

PHARMACOKINETICS/TOXICOKINETICS:

1) **Plasma Profiles with HMR 3647 and Clarithromycin in Rat Models with Hepatic Injury Induced by Carbon Tetrachloride and 1-Naphthylisothiocyanate; LPK-98-012.** This study was conducted by HMR, Saitama, Japan and was initiated on July 30, 1998.

The purpose of this study was to investigate the *in vivo* fate of HMR 3647 and clarithromycin (CAM) in hepatic injury. The hepatic injury was induced by carbon tetrachloride (causing a non-specific decrease in hepatic function) and 1-naphthylisothiocyanate (1-NAIT) (causing inhibition of biliary excretion).

Male Sprague-Dawley rats were used in this study (5 animals/group).

Results: Blood chemistries taken after injurious agents showed significant alterations in AST, and ALT. After injection of HMR 3647 in both models [24 hours after injury], plasma profiles showed ~1.5x increased t 1/2β, 0.6x total clearance and increased AUC ~1.6x when compared to controls.

After injection of CAM, results were similar (1.9x increased t 1/2β, 0.5x clearance and 2.2x AUC when compared to controls) for the carbon tetrachloride-treated animals. For the 1-NAIT-treated animals, the t 1/2β was 1.6x longer, clearance was 0.8x, and the AUC was 1.2x.

The sponsor concluded that the results were expected as HMR 3647 is not metabolized by the liver and is eliminated by biliary excretion and CAM undergoes extensive hepatic metabolism.

TOXICOLOGY:

1) **Single Dose Toxicity Study of HMR 3647 Administered Orally to Beagle Dogs; Study SBL-7891.** This study was conducted by [redacted] and was initiated on June 22, 1999. A GLP compliance statement is included in the report.

In this acute toxicity study, HMR 3647 was administered orally via capsule to 1 beagle (aged 7 months) /sex at 1000 or 2000 mg/kg. Animals were observed for 2 additional weeks and underwent gross necropsy.

Results: Vomition was reported in all of the animals. Abnormal stools were reported on Day 2, including mucus, occult blood and/or green stools.

A prolongation of the QTc interval was noted in the 1000 mg/kg animals (peaking at 6 hours), but the sponsor considered these findings incidental as they were not reported in the high dose animals. The sponsor concluded that the lethal dose of HMR 3647 was >2000 mg/kg when administered as a single oral dose to dogs.

2) **Antigenicity Study of HMR 3647; SBL 78-92.** This study was conducted by [redacted] and was initiated on July 22, 1999. A GLP compliance statement is included in the report.

In this study, five BALB/cAnNCrj mice/group were induced orally 5x/week for 3 weeks with HMR 3647 at 10 or 50 mg/kg, or i.p. with aluminum hydrochloride gel once/week for 3 weeks. The positive controls received ovalbumin i.p. once/week for 3 weeks. The negative controls received physiologic saline orally 5x/week for 3 weeks.

Passive sensitization: 2 Crj:CD (SD) rats were injected intracutaneously with various dilutions of sera from sensitized mice into 6 sites. Forty-eight hours later, the rats received 1 mL of challenge antigen mixed with 1% Evans blue into a tail vein.

Results: None of the rats injected with sensitized sera showed any 'colored spots' (indicating "passive cutaneous anaphylactic reaction") after challenge. All positive controls developed "positive colored spots" after challenge with ovalbumin.

The sponsor concluded that HMR 3647 has no antigenic properties under the conditions of this study.

RECOMMENDATIONS:

Internal comments: No action is indicated for this portion of the submission
External Recommendations (to sponsor): None for this portion of the submission

Reviewer signatur[redacted]

cc: list
HFD-520/Orig NDA
HFD-520/MO/Davidson
HFD-520/Chem/Yu
HFD-520/PT/Peters
HFD-520/Stat/Rochester
Draft date (# of drafts): 9/6/00; #1
HFD-520/CSO/Cintron

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

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: 6/9/00
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.

Studies reviewed within this submission:

1) *In Vitro* Study of HMR 3647 on Phosphodiesterase Activities; Study #98/10513/PH
2) Assessment of Local Anesthetic Potential of HMR 3647 After Topical Application (Corneal Reflex) in Guinea Pigs; Report #96/8857/PH
3) Electrophysiological Effects of HMR 3647 on Isolated Rabbit Purkinje Fibers; Report #96/8774/PH
4) Effect of Intravenous Administration of HMR 3647 on Respiration in Anesthetized Guinea Pigs; Report #97/9454/PH
5) Effect of Oral Administration of HMR 3647 on Diuresis in Rats; Report #96/8842/PH.
6) Effect of I.V. Administration of HMR 3647 on Diuresis in Saline-Loaded Rats; Report #97/9529/PH
7) Topical Anti-Inflammatory Activity of HMR 3647 on Croton Oil-Induced Ear Edema in Mice; Report #98/10214/PH

8) A Single Dose Oral Toxicity Study of HMR 3647 in Young Rats; Study No: — 78-83

9) A Single Dose Oral Toxicity Study of HMR 3647 in Young Dogs; Study No: — 78-81

10) 13 Weeks Oral Toxicity Study of HMR 3647 in the Rat; Study No: 96/9255/TX

11) Oral 6 Month Toxicity Study of HMR 3647 in Rats with a Four Week Recovery Period; Study No: 99/0273

12) Two Week Preliminary Toxicity Study of HMR 3647 by Intravenous Route in Rats; Study No: 14704 TSR

13) Changes in the Plasma Concentrations of HMR 3647 after 1 or 15 Oral Administrations of 100, 400, or 1000 mg/kg During a Preliminary Toxicology Study in the Dog; Study No: 96/8758/CN. The in vivo portion is reported as 96/8484/TX.

14) 13 Week Toxicity Study of HMR 3647 by Oral Administration (Capsules) in Beagle Dogs Followed By a 12 Week Recovery Period; Study No: 14869/TCC

15) Study Title: Toxicokinetics of HMR 3647 for Study 14688 TSC: Four Week Toxicity Study by I.V. Route in the Beagle Dog; Study No: 97/9903/CN

16) Pharmacokinetics of HMR 3647 in the Young Dog After One or Ten Oral Administrations of 100 mg/kg Study No: 98/10420/CN.

17) A Preliminary 2 Week Repeated Dose Toxicity Study of HMR 3647 Administered Orally to Young Beagle Dogs; Study No: — 78-82

18) 15 Day Oral (Gavage) Preliminary Toxicity Study of HMR 3647 in the Cynomolgus Monkey; Study No: 1453-552-036

19) 28 Day Oral (Gavage) Administration Subchronic Toxicity Study of HMR 3647 in the Cynomolgus Monkey; Study No: 1485-552-037

20) 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

21) 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

22) Nephrotoxicity Study of HMR 3647 After Single Oral Administration in the Rabbit; Study No: 97/9744/TX

23) Nephrotoxicity Study of HMR 3647 Given Orally in a Single Dose to Renally Impaired Wistar Rats; Study No: — 78-80

24) Serum Concentrations of HMR 3647 Observed During the Study Entitled: Nephrotoxicity Study of Dose in Renally Impaired Wistar Rats; Study No: 98/10642

25) Preliminary Two Week Ototoxicity Study with HMR 3647 by Oral Route (Gavage) to Rats; Study No: 17920 TSR

26) Four Week Ototoxicity Study with HMR 3647 By Oral Route (Gavage) to Rats; Study No: 18116 TSR

27) Antigenicity Study of HMR 3647 in Guinea Pigs: Active Systemic Anaphylaxis and Passive Cutaneous Anaphylaxis Tests; Study No: 016631

28) 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

29) Toxicokinetics of HMR 3647 during the study entitled: Study for effects of HMR 3647 on embryo-fetal development by oral administration (gavage) in rabbits as Study No: 15277 RSL; 97/9881/CN

30) 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

31) 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

32) In Vitro Mammalian Chromosome Aberration Test with HMR 3647 in Cultured Human Lymphocytes with Monkey Liver S9; Study No: 18861 MLH

33) In Vitro Mammalian Cell Gene Mutation Test with HMR 3647 in L5178Y TK± Mouse Lymphoma Cells without and with Monkey Liver S9; Study No: 18860 MLH

34) Study of the Absorption and Absolute Bioavailability of HMR 3647 in the Male Swiss Mouse After Single Intravenous or Oral Administration of 10 mg/kg of ^{14}C-HMR 3647; Study 97/9468/CN

35) Dose Proportionality Study After A Single Oral Administration of 5, 10 or 20 mg.kg/L of ^{14}C-HMR 3647 in the Male Sprague-Dawley Rat; Study #97/10175/CN

36) Study of the Absorption and Absolute Bioavailability of HMR 3647 in the Male Beagle Dog After Single Intravenous or Oral Administration of 5 mg/kg of ^{14}C- HMR 3647; Study #96/9162/CN.

37) Study of the Intestinal Absorption Sites of HMR 3647 in the Rat; Study #98/10426/CN

38) Gastrointestinal Absorption of ^{3}H- HMR 3647 in the Rat In Situ Intestinal Model; Study #97/9821/CN.

39) Quantitative Tissue Distribution of Radioactivity 1, 2, 6, 24, 48, and 72 h After Oral Administration of A Single Dose of 10 mg/kg of ^{14}C- HMR 3647 in the Male Albino Sprague-Dawley Rat; Study #97/9827/CN.

40) Tissue Distribution of Radioactivity by Autoradioluminography for 1 Year After Administration of A Single Oral Dose of 10 mg/kg of ^{14}C- HMR 3647 in Male Pigmented Long Evans Rat; Study #97/9822/CN.

41) Concentrations of Radioactivity in Myocardial Tissue 2, 6, 24, 72, and 96 Hours After 10 Daily Oral Administrations of 10 mg/kg of ^{14}C- HMR 3647 in Male Albino Sprague Dawley Rat; Study #98/10243/CN.

42) Tissue Distribution of Radioactivity by Autoradioluminography 2, 6, and 24 Hours After Administration of A Single Oral Dose of 10 mg/kg of ^{14}C-HMR 3647 to 12 and 18 Day Pregnant Female Albino Sprague-Dawley Rat; Study #97/10059/CN.

43) Tissue Distribution of Radioactivity by Autoradioluminography 24, 72, 168, and 312 hours After Administration of A Single Oral Dose of 10 mg/kg of ^{14}C-HMR 3647 and 4 Comparators (^{14}C-HMR 3004, ^{14}C-Roxithromycin, ^{14}C-Clarithromycin and ^{3}H- Azithromycin) In Male Pigmented Long Evans Rat; Study #97/9596/CN

44) *In vitro* Biotransformation of HMR 3647 by Male and Female Mouse Hepatocytes; Study #97/9419/CN

45) Metabolic Profiles and Quantitation of the Metabolites of HMR 3647 in Plasma, Urine and Feces Following a Single Oral Administration of 10 mg/kg of ^{14}C-HMR 3647 to Male Rats; Study #98/10759/CN

46) Metabolic Profiles and Quantification of the Metabolites of HMR 3647 in Plasma, Urine and Feces Following Single Oral Administration of 5 mg/kg of ^{14}C-HMR 3647 to Male Dogs; Study #98/10758/CN

47) Urinary and Fecal Excretion of Radioactivity after a Single Intravenous or Oral Administration of 10 mg/kg of ^{14}C-HMR 3647 in the Male and Female Sprague-Dawley Rat; Study #96/8801/CN

48) Biliary Excretion and Enterohepatic Circulation of Radioactivity in the Conscious Male Sprague-Dawley Rat After A Single Intravenous Administration of 10 mg/kg ^{14}C-HMR 3647; Study #97/10103/CN

49) In Vitro Effects of HMR 3647 on Hepatic Cytochromes P-450 From Male Rats- Comparison with TAO; Study #97/9951/CN

51) Potential Cytochrome P450 Interaction Studies Using Rat Liver Microsomes with HMR 3647; Study #98/10225/CN

52) In Vivo Effects of HMR 3647 on Hepatic Cytochromes P-450 From Male Rats- Comparison with TAO; Study #97/9952/CN
Scientific literature reviewed: Yes

PHARMACOLOGY:
In Vitro Study of HMR 3647 on Phosphodiesterase Activities; Study #98/10513/PH. This study was conducted by [redacted]. "The work was planned, conducted and reported within the spirit of Good Laboratory Practice." The study was initiated on June 29, 1998.

- species/strain: Male Swiss mice
- age: 6 weeks at study initiation
- weight: 30 gms
- dosage groups in administered units: 10 mg/kg
- route, form, volume, and infusion rate: i.v. or p.o. at 2 mL/kg in an aqueous solution of 2.5 mM HCl.

Drug, lot: Unlabeled Batch 9 or labeled Batch X10509A

The study was performed to demonstrate the *in vitro* effects of HMR 3647 on phosphodiesterase I from bovine brain and phosphodiesterases II, III, IV, and V from human cells.

Doses tested: 1, 10 and 100 μm. Appropriate reference compounds were tested simultaneously to validate the experiment.

Results: Phosphodiesterase I and II activities were inhibited in a dose-related fashion (88% at 100 μm). Phosphodiesterase III and V activities were inhibited >50%, but IV was inhibited <38%.

Effects on coagulation in rats and rabbits were tested. At 15 mg/kg i.v. in rats, a significant increase in APTT was noted in 2 experiments. No effects were appreciated at 5 mg/kg. In rabbits, HMR 3647 inhibited arachidonate-induced aggregation and collage and ADP-induced aggregation thus it appears to have antiaggregatory activity *in vitro*. When co-administered with aspirin, there was a strong potentiation of the anti-aggregant activity. The effects on thromboxane synthetase and thromboxane TxA_2 were evaluated in rabbit platelets. A significant inhibitory effects on thromboxane synthetase was reported, but a very weak effect on thromboxane receptor sites. Another study with rabbit platelets tested the ability of HMR 3647 to inhibit COX_1. No inhibition was reported.

Assessment of Local Anesthetic Potential of HMR 3647 After Topical Application (Corneal Reflex) in Guinea Pigs; Report #96/8857/PH. This study was conducted by HMR, Cedex, France. It was carried out "along the lines of the GLP".
In this study, 5 male Dunkin Hartley guinea pigs/group were treated with 0.1, 0.5 or 1.0% concentration of HMR 3647 in the left eye and the right eye served as the controls.
Results: At 1%, slight suppression of the corneal reflex was noted. Additionally, the HMR 3647-treated eye 'appeared more sensitive to stimulation' and lacrimation was observed.

SAFETY PHARMACOLOGY:

Cardiovascular effects:
Electrophysiological Effects of HMR 3647 on Isolated Rabbit Purkinje Fibers; Report #96/8774/PH
This study was reviewed by HFD-110. Their conclusions were as follows: "HMR 3647 lengthened cardiac APD_{90} potential of rabbit Purkinje fibers in a concentration-related manner. Increases were similar to those observed with erythromycin and clarithromycin. While the absolute changes in APD_{90} were greater at 0.2 Hz than at 1.0 Hz, the percent increases in APD_{90} were similar at these two stimulation rates (due to baseline APD_{90} being higher at 0.2 Hz than at 1.0 Hz). Therefore, HMR 3647's effects on APD_{90} were not inverse-rate dependent; this finding contrasts with the inverse rate-dependency noted with selective IKr blocking drugs such as dofetilide."

Overall, HFD-110's conclusions for the preclinical cardiovascular studies were as follows: "Preclinical studies with HMR 3647 demonstrated a potential to affect ventricular repolarizations, i.e.prolong QT or QTc intervals. HMR 3647 inhibited two ionic currents, HERG and IKs, involved in ventricular repolarization, lengthened action potential duration in rabbit cardiac Purkinje fibers, and prolonged QTc (Bazett and Fredericia) in anesthetized and conscious dogs given this drug intravenously and/or orally. Effects on QTc were observed with both acute and 30 day dosing regimes. Although HMR 3647 did not affect absolute QT interval, it increased heart rate. An absence of effect on absolute QT interval in the presence of a heart rate increase strongly supports the conclusion of a drug-related effect on ventricular repolarization since in the absence of drug, a heart rate increase should shorten QT. All of the above-mentioned effects were concentration or dose related. HMR 3647's potencies on ionic currents and action potential duration were similar to those seen with erythromycin, which is known to prolong QTc interval and induce *torsade de pointes* in humans."

Pulmonary effects:
1) Effect of Intravenous Administration of HMR 3647 on Respiration in Anesthetized Guinea Pigs; Report #97/9454/PH. This study was conducted by Hoechst Marion Roussel, Cedex, France. A GLP compliance statement is included in the report. The study was initiated on 2/17/97.

Doses administered: vehicle (isotonic mannitol and water), 5 or 15 mg/kg HMR 3647 administered i.v.
Animals: Male Dunkin Hartley guinea pigs- n= 6, 5 and 7 for the respective groups.
Results: No significant differences from controls were noted for up to 60 minutes post-dosing.
Renal effects:

2) Effect of Oral Administration of HMR 3647 on Diuresis in Rats; Report #96/8842/PH.
3) Effect of I.V. Administration of HMR 3647 on Diuresis in Saline-Loaded Rats; Report #97/9529/PH.

These studies were conducted by HMR, Cedex, France and a GLP compliance statement is included.

Doses administered: vehicle (0.5% methylcellulose), 30, 100 or 300 mg/kg for the oral study and 5 or 15 mg/kg i.v.
Animals: Male CD rats, 10/group
Results: Effects on diuresis (decreased volume), electrolytes (increased Na/K, decreased Mg excretion), creatinine, urea, and urinary volume were noted in the mid and high dose groups in the oral study. In the i.v. study, no significant effects were appreciated.

Gastrointestinal effects:

1) Effect of Oral Administration of HMR 3647 on the Digestive System in Rats; Report #96/8841/PH.
2) Effect of Intravenous Administration of HMR 3647 on the Digestive System in Rats; Report #97/9528/PH. These studies were conducted by HMR, Cedex, France. GLP compliance statements are included in the reports.

Doses were 30, 100 and 300 mg/kg in male rats for the oral study and 5 and 15 mg/kg in the i.v. study.
Results: Oral study: HMR 3647 decreased gastric emptying time and gastric acidity at the mid and high doses, but had no effect on G.I. transit time. I.V. study: No G.I. effects were noted.

3) In Vitro Effect of HMR 3647 on Isolated Guinea Pig Ileum; Report #96/8844/PH. This study was conducted by HMR, Cedex, France. No GLP compliance statement is included in the report.

Isolated guinea pig ileum was tested with HMR 3647 at $5x10^{-4}$ to 10^{-6} M concentrations.
Results: There was a slight spasmogenic effect at $5x\ 10^{-4}$ and 10^{-4}M.

4) Study of the Intestinal Absorption Sites of HMR 3647 in the Rat; Study #98/10426/CN. This study was conducted by HMR, Cedex, France.

Male Sprague-Dawley rats (n=5) were killed and sections of intestine were removed. Ussing chambers were used in evaluate the absorption of the radiolabeled (^{3}H) test compound.
Results: The main absorption site was in the ileum, with duodenum, jejunum and colon having ~1/5 the amount absorbed.

5) Gastrointestinal Absorption of ^{3}H- HMR 3647 in the Rat In Situ Intestinal Model; Study #97/9821/CN.

This in vitro portion was conducted by HMR, Cedex, France and was initiated on 7/1/97.
Six groups of 4 male Sprague-Dawley rats had intestinal and mesenteric vein cannulations.

Formulation	HMR 3647 []	3H- HMR 3647	P-GP Substrate/Inhibitor
1	10^{-6} M	1.2 μCi/mL	None
2	10^{-4} M	1.2 μCi/mL	None
3	10^{-6} M	1.2 μCi/mL	Verapamil
4	10^{-4} M	1.2 μCi/mL	Verapamil
5	10^{-6} M	1.2 μCi/mL	Ketoconazole
6	10^{-4} M	1.2 μCi/mL	Ketoconazole

Substrates were used to determine the effect, if any, on appearance of unchanged drug in mesenteric plasma.
Results: Absorption in the jejunum markedly increased with an increase from 10^{-6} M to 10^{-4} M HMR 3647. Coperfusion with ketoconazole or verapamil increased absorption (~1.5x), demonstrating mediation by P-GP (P-glycoprotein 170). Approximately 55% of the total radioactivity was recovered after 90 minutes indicating intestinal metabolism in rats. Recovery of parent compound was increased with coperfusion was done, indicating metabolic competition.

Skin effects:
Topical Anti-Inflammatory Activity of HMR 3647 on Croton Oil-Induced Ear Edema in Mice; Report #98/10214/PH. This study was conducted by HMR, Cedex, France and a GLP compliance statement is included.
Doses: 0.1, 0.5, 1, or 2 mg/ear
Animals: CD male mice
Results: HMR 3647 elicited a significant decrease in ear edema in a dose-dependent fashion. The ED_{50} was estimated at 0.2 mg/ear.

PHARMACOKINETICS/TOXICOKINETICS:

1) Study of the Absorption and Absolute Bioavailability of HMR 3647 in the Male Swiss Mouse After Single Intravenous or Oral Administration of 10 mg/kg of ^{14}C-HMR 3647; Study 97/9468/CN. This study was conducted by HMR, Cedex, France and was initiated on 3/5/97. A GLP compliance statement is included in the report.
   - species/strain: Male Swiss mice

- age: 6 weeks at study initiation
- weight: 30 gms
- dosage groups in administered units: 10 mg/kg
- route, form, volume, and infusion rate: i.v. or p.o. at 2 mL/kg in an aqueous solution of 2.5 mM HCl.

Drug, lot: Unlabeled Batch 9 or labeled Batch X10509A

Observations and times:

Five mice were killed immediately after dosing at 0.05, 0.25, 0.30, 0.75, 1, 1.5, 2, 3, 4, 5, 6, 8, and 24 hours post-dosing for both routes of administration.

- Toxicokinetics: Samples were analyzed by HPLC and fluorescence detection. Limit of quantitation for HMR 3647 was [redacted], and for radioactivity was [redacted]

Results:

- Toxicokinetics: I.V. administration: The volume of distribution (elimination phase) was low at 0.043L. Total clearance was low at 0.024 L/hr. The t ½ was 1.2 hours in plasma. AUC (0-z) [area under the plasma concentrations vs. time curve] was approximately 2.4x higher with the i.v. administration compared to the oral administration.

Oral administration: Cmax was reached 1.5 hours after dosing with detectable concentrations remaining up to 8 hours post-dosing. Absorption and bioavailability were ~50% of the administered dose.

Key Study Findings: Irrespective of the route of administration, the plasma concentrations of HMR 3647 represented ~70% of the radioactivity, indicating moderate metabolism of the parent compound. A minimal first pass effect was reported.

2) Dose Proportionality Study After A Single Oral Administration of 5, 10 or 20 mg.kg/L of ^{14}C-HMR 3647 in the Male Sprague-Dawley Rat; Study #97/10175/CN. This study was conducted by HMR, Cedex, France and was initiated on 1/15/98.

- species/strain: Male Sprague Dawley rats
- age: 7 weeks at study initiation
- weight: ~200 gms
- dosage groups in administered units: 5, 10 or 20 mg/kg
- route, form, volume, and infusion rate: First portion of the study: 5 rats/group given a single oral dose of radioactive compound. Second portion of the study: 15 animals/group were given a single oral dose of radioactive compound.

Drug, lot: Unlabeled Batch 22 or labeled Batch X10509A

Toxicokinetics: First portion of the study: Samples were taken at 15, 30, and 45 minutes, and 1, 1.5, 2, 4, 6, 8 and 24 hours post-dosing. Second portion of the study: Samples were taken at 30 minutes, 2 and 6 hours post-dosing. Samples were pooled per dose and per sampling time.

First portion of the study:

Parameter	5 mg/kg	10 mg/kg	20 mg/kg
Cmax (mg.eq/L)	0.095	0.265	0.652
Tmax (hr)	0.25-4	0.25-4	0.25-6
AUC (0-z)	0.290	1.006	3.071

Second portion of the study:

Plasma Concentrations

Time	5 mg/kg	10 mg/kg	20 mg/kg
0.5 hr			
2 hr			
6 hr			

The plasma concentrations were irregular with 2 peaks (first portion of the study) at 15 minutes after dosing and 4-6 hours post-dosing. A non-linear profile was found (radioactivity increased more than dose). In the second portion of the study, HMR 3647 represented 60+% of the total radioactivity 6 hours after dosing. The terminal t ½ was 1.7 hours. Metabolites represented ~18% of the total radioactivity.

3) **Study of the Absorption and Absolute Bioavailability of HMR 3647 in the Male Beagle Dog After Single Intravenous or Oral Administration of 5 mg/kg of ^{14}C- HMR 3647; Study #96/9162/CN.** This study was conducted by HMR, Cedex, France and was initiated on 10/28/96.

- species/strain: Male beagle dogs
- age: 14-15 months
- weight: 12.8 kg
- dosage groups in administered units: 5 mg/kg
- route, form, volume, and infusion rate: Drug, lot: Unlabeled Batch 9 or labeled Batch X10509A

Toxicokinetics: Dogs were treated orally, then i.v. after a 4 week wash-out period. Plasma samples were taken pre-dosing, and at 0.83, 0.25, 1, 2, 3, 4, 6, 8, 24, 30 and 48 hours after dosing.

Results: After i.v. administration, the volume of distribution was ~6x the animals' body size and total clearance was high. The terminal t ½ was 2.3 hrs.
After oral administration, Cmax was reached before 2 hour test point. Absorption (83%) and bioavailability (54%) indicate a moderate first pass effect.
The main metabolite in the dog was the N-oxide-pyridine (RU 76584).
Approximately 1.5% of the administered radioactivity was still present 48 hours post-i.v. dosing and 3.2% post-oral dosing.

4) **Quantitative Tissue Distribution of Radioactivity 1, 2, 6, 24, 48, and 72 h After Oral Administration of A Single Dose of 10 mg/kg of ^{14}C- HMR 3647 in the Male Albino Sprague-Dawley Rat; Study #97/9827/CN.**
This study was conducted by HMR, Cedex, France and was initiated on 7/8/97 under GLP conditions.

Three male Sprague-Dawley rats/group were gavaged once with 10 mg/kg of ^{14}C-HMR 3647. Animals were killed at 1, 2, 6, 24, 48, and 72 hours post-dosing. Organs/tissues were sampled and radioactive content measured by liquid scintillation.

Results: Measured radioactivity decreased from 1-2 hours, increased from 2-6 hours, then decreased significantly from 6-24 hours. Stomach contents accounted for 4.1% of the dose at 2 hours. By 24 hours post-dosing, the large intestine had the highest radioactivity at 11%. The elimination t ½ was 3.4 hours for lungs, 7.9 hours for the brain and 10.2 hours for testes. More than 89% of the dose was accounted for between 1-2 hours post-dosing. After 48 and 72 hours, the highest radioactive contents were found in liver, kidneys, spleen, adrenals and mesenteric lymph nodes, but only 0.35% of the dose by 72 hours. However, a late elimination phase seemed to occur between 24 and 72 hours (tissue levels did not change appreciably during this time) but it did not seem to contribute significantly to the AUC.

Throughout the study, significant levels of radioactivity were found in the GI tract. The sponsor discounted contamination as the cause and proposed that "HMR 3647 (and its metabolites) is heavily secreted through the gastrointestinal tract walls."

5) **Tissue Distribution of Radioactivity by Autoradioluminography for 1 Year After Administration of A Single Oral Dose of 10 mg/kg of ^{14}C- HMR 3647 in Male Pigmented Long Evans Rat; Study #97/9822/CN.**
This study was conducted by HMR, Cedex, France, and was initiated on 7/1/97 under GLP conditions.

Three male Long Evans rats/group were gavaged once with 10 mg/kg of ^{14}C-HMR 3647. Animals were killed at 3, 14, 28, 42, and 56 days post-dosing and one/dose group was killed at 6, 9 and 12 months post-dosing. Pigmented skin and uveal tract were evaluated for radioactivity.

Results: In the uveal tract, radioactivity persisted in pigmented tissues for 12 months. In pigmented skin, no significant radioactivity was appreciated at any time. The sponsor supposed that HMR 3647 and/or its metabolites bound to melanin in the eye and that was why radioactivity persisted for 12 months.

6) **Concentrations of Radioactivity in Myocardial Tissue 2, 6, 24, 72, and 96 Hours After 10 Daily Oral Administrations of 10 mg/kg of ^{14}C- HMR 3647 in Male Albino Sprague Dawley Rat; Study #98/10243/CN.**
This study was conducted by HMR, Cedex, France, and was initiated on 2/27/98 under GLP conditions.

Three male Sprague-Dawley rats were treated for 10 days with gavage doses of 10 mg/kg of ^{14}C-HMR 3647. Animals were killed at 2, 6, 24, 72 and 96 hours post-dosing. Samples were taken of blood, plasma, atria and ventricles. Radioactivity was measured by liquid scintillation.

Results: In atria and ventricles, levels of radioactivity were 4-8x higher than in plasma. At the 2, 6 and 24hour timepoints, concentrations in blood were ~ equal to those in plasma, but were significantly higher (2-4x) at later timepoints. There was no apparent increase in plasma concentrations over time.

7) Tissue Distribution of Radioactivity by Autoradioluminography 2, 6, and 24 Hours After Administration of A Single Oral Dose of 10 mg/kg of ^{14}C-HMR 3647 to 12 and 18 Day Pregnant Female Albino Sprague-Dawley Rat; Study #97/10059/CN. This study was conducted by HMR, Cedex, France, and was initiated on 11/19/97 under GLP conditions.

One pregnant rat/timepoint (2, 6 or 24 hours post-dosing) was killed after a single dose of ^{14}C-HMR 3647 on Day 12 or 18 of pregnancy. However, only 1/3 of the animals was pregnant on Day 12 so only the 2 hour sample was analyzed.

Results: Levels of radioactivity were highest in the liver, kidney and spleen. A low level remained in the GI tract at 24 hours, but most other tissues had undetectable levels. The feti from the Day 18 dams had small amounts of radioactivity at 6 hours (liver and GI tract) but none detectable at 24 hours post-dosing.

8) Tissue Distribution of Radioactivity by Autoradioluminography 24, 72, 168, and 312 hours After Administration of A Single Oral Dose of 10 mg/kg of ^{14}C-HMR 3647 and 4 Comparators (^{14}C-HMR 3004, ^{14}C-Roxithromycin, ^{14}C-Clarithromycin and ^{3}H- Azithromycin) In Male Pigmented Long Evans Rat; Study #97/9596/CN. This study was conducted by HMR, Cedex, France, and was initiated on 4/8/97 under GLP conditions.

Four male Long Evans rats/group were dosed once by gavage with 10 mg/kg of the radioactive compound. Animals were killed at 24, 72, 168 or 312 hours post-dosing and the eyes and pigmented skin and whole bodies were frozen, embedded and sectioned for imaging analysis.

Results: High levels of radioactivity were found at all timepoints with HMR 3647 in the uveal tract, but minimal amounts were found in pigmented skin. At 312 hours, radioactivity persisted in the uveal tract. For the comparators, HMR 3004 had persistent radioactivity in the uveal tract, with minimal amounts in liver, kidney and pigmented skin at 312 hours. Roxithromycin- and Clarithromycin-associated radioactivity persisted in the uveal tracts at 312 hours. For Azithromycin, the uveal tract retained much of the radioactivity at 312 but less than at the 168 hour time point. Additionally, significant amounts of radioactivity were reported in the GI tract at 24 hours. However, Azithromycin was labeled with tritiated thymidine so it is difficult to relate these data with the other 'comparators'.

9) *In vitro* Biotransformation of HMR 3647 by Male and Female Mouse Hepatocytes; Study #97/9419/CN. This study was conducted by HMR, Cedex, France, and was initiated on 2/19/97 under GLP conditions.

In this study, isolated male and female mouse hepatocytes (1 mouse/sex) were incubated with 0.5μg/mL of ^{3}H-HMR 3647 for 0.083, 0.25, 0.5, 1, 2, 3, and 24 hours to evaluate the rate of metabolism and metabolic profiles of HMR 3647. The extracellular media and cells were separately collected and pooled. Metabolic profiles were determined by [redacted] in extracellular media and cell homogenates after 24 hours of incubation. Extracellular media were also evaluated by [redacted]

Results: The parent compound after 3 hours of incubation composed 71-81% of the radioactivity. Female hepatocytes had a higher rate of metabolism than males (parent compound was 28% from the females and 50% from the males after 24 hours of incubation).

Eight metabolic peaks were identified at 24 hours with the primary ones being:
1) M8 – the N-oxide-pyridine derivative (~7% in males, 16% in females)
2) M14 – the N-desmethyl-desosamine derivative (~8% in males, 13% in females)
3) M2/M3 – mixture of metabolites including the acid resulting from the loss of aryl rings

Additional metabolites were present in lesser amounts (M5- N-desmethyl-desosamine and M11- the alcohol). Neither glucuro- nor sulfo-conjugation was observed.

10) Metabolic Profiles and Quantitation of the Metabolites of HMR 3647 in Plasma, Urine and Feces Following a Single Oral Administration of 10 mg/kg of ^{14}C-HMR 3647 to Male Rats; Study #98/10759/CN. This study was conducted by HMR, Cedex, France and was initiated on 12/15/98 under GLP conditions.

Four male Sprague-Dawley rats/group were gavaged with a single 10 mg/kg dose of ^{14}C-HMR 3647. Groups were euthanized at pre-dosing and at 0.25, 0.75, 1, 2, 4, and 8 hours after dosing. Excreta were collected from another group of 4 rats at 0-24 hours and 24-48 hours post-dosing.
Metabolic profiles were determined in pools of plasma, urine and feces by radiometry, mass spectroscopy and after purification (plasma and feces) and directly (urine).

Results: Parent compound accounted for 72% of the plasma radioactivity, 4% of the dose in urine and 53% of the dose in feces. The N-desmethyl-desosamine derivative was the main metabolite in feces (~7% of the dose), and 3% of the dose were the alcohol and the acid, respectively, resulting from the loss of the aryl rings. Urinary metabolites were numerous but none were >0.5% of the dose.

11) Metabolic Profiles and Quantification of the Metabolites of HMR 3647 in Plasma, Urine and Feces Following Single Oral Administration of 5 mg/kg of ^{14}C-HMR 3647 to Male Dogs; Study #98/10758/CN. This study was conducted by HMR, Cedex, France and was initiated on 2/16/99 under GLP conditions.

Three male beagle dogs were gavaged with a single 5 mg/kg dose of ^{14}C-HMR 3647. Dogs were sampled at pre-dosing, 0.25, 1, 4, 8 and 24 hours post-dosing (plasma), urine and feces at 0-24, 24-48, and 48-72 hours post-dosing.

Results: The AUC represented ~70% of the total plasma radioactivity. The main metabolites were the N-oxide pyridine (15.3%) and the N-desmethyl-desosamine (2.7%). No plasma radioactivity was reported 24 hours post-dosing. The majority (>95%) of urinary excretion occurred within 24 hours of dosing. Fecal excretion was 42% by the end of 24 hours post-dosing.
Parent compound was 46% of urine radioactivity and 35.8% of feces radioactivity. The N-oxide pyridine was 1.2% of the urinary excretory product and the N-desmethyl-desosamine was 17.7% of the fecal excretory product.

12) Urinary and Fecal Excretion of Radioactivity after a Single Intravenous or Oral Administration of 10 mg/kg of ^{14}C-HMR 3647 in the Male and Female Sprague-Dawley Rat; Study #96/8801/CN. This study was conducted by HMR, Cedex, France and was initiated on 6/11/96 under GLP conditions.
Ten Sprague-Dawley rats/sex were divided into 2 groups of 5 each. One group received their dose by gavage, one group by i.v. administration into a caudal tail vein. Urine and feces were collected in 24 hour periods for 4 days post-dosing. Portions of the carcass were minced and homogenized.

Results: Minimal radioactivity remained in the carcasses after 4 days (<1%). Excretion was primarily by the fecal route (~80%) and was rapid (most excreted within 24 hours). The rate of elimination was slower in females than males after i.v. dosing, but comparable after p.o. dosing. Urinary excretion was higher after i.v. dosing (~13.4% of dose) than after p.o. dosing (~6%).

13) Biliary Excretion and Enterohepatic Circulation of Radioactivity in the Conscious Male Sprague-Dawley Rat After A Single Intravenous Administration of 10 mg/kg ^{14}C-HMR 3647; Study #97/10103/CN. This study was conducted by HMR, Cedex, France and was initiated 11/17/97 under GLP conditions.

Five donor rats were given a single radioactive dose of 10 mg/kg i.v. Enterohepatic circulation was interfered with and bile was collected hourly at 0-6 hours and 6-24 hours post-dosing. Four recipient rats were administered intraduodenally the pooled radioactive bile (2.4 mg eq/kg) from the 0-4 hour collections and bile was collected hourly from 0-6 hours and from 6-24 hours post-dosing.

Results: Bile was the major route of elimination in the donor rats (57% of the dose within 24 hours). Fecal excretion (0-24 hours) accounted for ~16% of the dose and was primarily unchanged parent compound (41% of the 0-4 hour

pool). The N-oxide-pyridine derivative was the main metabolite in bile (36% of the 4-24 hour pool). Urinary excretion accounted for ~19% of the dose. Parent compound represented 87% of this radioactivity.

In the recipient rats, biliary excretion accounted for ~11% of the administered dose. Parent compound accounted for 29% of the radioactivity in the 0-6 hour collection.

14) In Vitro Effects of HMR 3647 on Hepatic Cytochromes P-450 From Male Rats- Comparison with TAO; Study #97/9951/CN. This study was conducted by HMR, Cedex, France and was initiated in September, 1995 under GLP conditions.

The comparator was troleandomycin (TAO), a recognized inhibitor of CYP3A (inducible by glucocorticoids) and is commonly involved with drug-drug interactions. T ½ of many drugs metabolized by CYP3A is prolonged when co-administered with TAO (ergotamine, theophylline).

Four male Sprague-Dawley rats were pre-treated with dexamethasone and livers were removed. Doses of HMR 3647 tested were 1μM to 100μ. Doses of TAO tested were 2μM to 40 μM.

Results: HMR 3647 and CYP active site showed a Reverse Type I interaction with no nitrosoalkane complex. This should lead to a slow metabolism of HMR 3647. The TAO and CYP active site showed a Type I interaction with a high nitrosoalkane complex. This should provide a rapid substrate metabolism.

15) Potential Cytochrome P450 Interaction Studies Using Rat Liver Microsomes with HMR 3647; Study #98/10225/CN. This study was conducted by HMR, Cedex, France and was initiated in March 1997 under GLP conditions.

Biological material was derived from a pool of 15 control male rat livers, 15 3-methylcholanthrene pre-treated male rat livers and 15 dexamethasone pre-treated male rat livers. The following P450 enzymes were evaluated: Ethoxyresorufine O-deethylase activity, testosterone hydroxylase activity.

Results: Testosterone hydroxylase activity (6βOHT, 2βOHT, 15βOHT) was inhibited in controls with HMR 3647. 7∝OHT, 16∝OHT and androstendione were not affected by HMR 3647.

Dexamethasone pre-treatment showed weak inhibition of testosterone hydroxylase at doses up to 5 mM where strong inhibition was noted.

3-Methylcholanthrene inhibited ethoxyresorufine O-deethylase activity.

The sponsor concluded that the most likely enzyme for interaction with HMR 3647 was CYP3A on the basis of 15OHT inhibition. HMR 3647 had a strong interaction with CYP1A1 at all dose levels tested. They expressed concern that saturation/inhibition of the CYP3A pathway might shift the metabolism of HMR 3647 to CYP1A1. The clinical significance of this shift is unclear.

16) In Vivo Effects of HMR 3647 on Hepatic Cytochromes P-450 From Male Rats- Comparison with TAO; Study #97/9952/CN. This study was conducted by HMR, Cedex, France and was initiated in October 1995 under GLP conditions.

Livers from 5 male Sprague-Dawley rats were homogenized after five days of dosing with HMR 3647 or TAO or sesame oil alone. Pre-treated rats were dosed with dexamethasone once/day for 3 days. Evaluation was performed for nitrosoalkane complexes, erythromycin N-demethylase activity and CPY3A by western blot technique.

Results: No P-450 complexes were formed after 5 days of dosing with HMR 3647 compared to 67.5% complex formation after 5 days of TAO dosing. In dexamethasone pre-treated rats, 0.7% complexes were formed after 1 dose of HMR 3647 while 51% complexes were formed after 1 dose of TAO.

Erythromycin N-demethylase activity: activity was increased 3x with HMR 3647, 7x with dexamethasone and 10x with TAO.

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

The sponsor concluded that HMR 3647 did not generate significant inhibitor complexes in the rat, and thus should not be considered a significant inducer of CYP3A.

TOXICOLOGY:

Study Title: A Single Dose Oral Toxicity Study of HMR 3647 in Young Rats
Study No: ~ /8-83
Vol #, and page #: 12, pg. 213
Conducting laboratory and location: —
Date of study initiation: 10/27/98
GLP compliance: Yes
QA- Report: No
Methods:
Dosing: Based upon a HMR study where 2000 mg/kg did not elicit death or significant clinical signs.

- species/strain: Crj:CD(SD) IGS rats
- #/sex/group or time point: 8 except the 2000 mg/kg group where there were 4.
- age: 5 days of age
- weight: 8.6- 14.0 g
- dosage groups in administered units: 0, 125, 250, 500, 1000 or 2000 mg/kg/d as a single dose
- route, form, volume, and infusion rate: Oral by gavage

Drug, lot: Batch 22 at —purity
Formulation/vehicle: 0.5% methylcellulose solution
Observations and times:

- Clinical signs: Pre-dosing and twice/day during observation period
- Body weights: Pre-dosing and on Days 1, 4, 8 and 13
- Gross pathology: All animals
- Organs weighed: None
- Histopathology: Lungs of dead animals from 500 mg/kg group, livers from surviving animals in the 250 mg/kg group and skin from 'unkempt' animals from the 250 mg/kg group. Liver, kidneys, and abnormal tissues from all animals were examined. Livers from 2/sex from the control group were examined histologically.

Results:

- Clinical signs: All of the 2000 mg/kg animals died within 5 hours of dosing. All of the 1000 mg/kg animals died, all but 2 males on Day 1. Death occurred in 5 males and 6 females from the 500 mg/kg group within 24 hours of dosing. From the 250 mg/kg group, 3 males and 3 females died within 24 hours of dosing.

Antemortem signs included decreased activity, skin blanching, and disappearance of the righting reflex and hypothermia.

- Body weights: No significant treatment-related differences from controls were noted.
- Gross pathology: The only significant lesion reported was dark coloration of the liver in 2 males and 1 female from the 250 mg/kg group.
- Histopathology: No significant treatment-related lesions were reported. No individual histopathology sheets were provided.

The sponsor concluded that 250 mg/kg was the "approximate lethal dose level of HMR 3647 in young rats under the conditions of this study."

Study Title: A Single Dose Oral Toxicity Study of HMR 3647 in Young Dogs
Study No: —78-81
Vol #, and page #: 13, pg. 2
Conducting laboratory and location: —
Date of study initiation: 10/28/98

GLP compliance: Yes
QA- Report: Perhaps but not translated from the Japanese so unable to accurately determine
Methods:
Dosing:

- species/strain: beagle dogs
- #/sex/group or time point: 1
- age: 21 days of age at dosing
- weight: 0.89-1.18 kg on day of dosing
- dosage groups in administered units: 0, 500, 1000 or 2000 mg/kg
- route, form, volume, and infusion rate: Single dose by gelatin capsule

Drug, lot: Batch 22
Results:

- Clinical signs: The high dose female died 2 hours post-dosing and the mid dose male died approximately 4 hours post-dosing. In surviving animals, signs included vomition (all dose groups), decreased spontaneous activity (all dose groups), tremor (all dose groups), tachypnea (high dose only), mydriasis, cyanosis and/or hypothermia.
- Body weights: No significant differences from controls were appreciated.
- Gross pathology: The premature decedent from the 1000 mg/kg group showed congestion in the SI.
- Histopathology: The premature decedent from the 1000 mg/kg group had inflammatory cell infiltrates, hemorrhage and necrosis of the Peyer's patches.

Key Study Findings: The sponsor concluded that the lethal dose of HMR 3647 was >1000 mg/kg when administered as a single dose orally to young dogs.

Study Title: <u>13 Weeks Oral Toxicity Study of HMR 3647 in the Rat</u>
Study No: 96/9255/TX
Vol #, and page #: 17, pg. 2
Conducting laboratory and location: HMR Drug Safety, Cedex, France
Date of study initiation: 12/10/96
GLP compliance: Yes
QA- Report: Yes
Methods:
Dosing: Doses chosen from the results of the 30 day study where 50 mg/kg was considered the NOAEL.

- species/strain: Sprague-Dawley OFA rats
- #/sex/group or time point: 15
- dosage groups in administered units: 0 (0.5% methylcellulose), 20, 50, or 150 mg/kg/d
- route, form, volume, and infusion rate: Gavage at 10 mL/kg

Drug, lot: Batch 14
Observations and times:

- Clinical signs: Three times/day except on weekends
- Body weights: Twice pre-dosing and weekly thereafter
- Food consumption: Groups of 3/cage
- Ophthalmoscopy: Pre-dosing and at the end of dosing period
- Hematology: Day 91
- Clinical chemistry: 24 hours after the last dose, including fecal occult blood
- Urinalysis: End of dosing in all animals from metabolism cages
- Gross pathology: All animals
- Organs weighed: Adrenals, brain, cecum (filled and empty), heart, kidneys, liver, lungs, ovaries, pituitary, prostate, seminal vesicles, spleen, testes, thymus, thyroids, uterus
- Histopathology: From controls and high dose animals, and target organs from mid and low dose groups.
- Toxicokinetics: Not performed

Results:

- Clinical signs: Alopecia and broken vibrissaes were noted in dose-dependent increasing frequencies (alopecia in 3 females at 20 mg/kg/d, 2 males and 6 females at 50 mg/kg/d and 4 males and all females at 150 mg/kg/d), primarily in females (from study initiation) and in males from Day 36 until the end of the study. "These observations were also observed in some control animals (alopecia in 2 males and 1 female)

with a similar pattern of that observed at the 20 and 50 mg/kg/d doses. Therefore, no treatment related effect can be confirmed at these doses." (Sponsor conclusion). However, since there is a dose-dependent increasing frequency and females were consistently more affected than males, it is considered a treatment-related effect.

Additionally, ptyalism was reported in all dose groups, but no significant effect on feed consumption or body weights were noted.

- Body weights: The high dose males had a –7% body weight gain when compared to controls with a 12% delay in body weight gain over the length of the study.
- Food consumption: Comparable across groups
- Ophthalmoscopy: No treatment-related differences from baseline were appreciated.
- Hematology: An increase in eosinophil numbers [~3x] (absolute and relative) was reported for the high dose animals.
- Clinical chemistry: ALTs were increased in high dose animals (3.6x in males, 2.6x in females). ASTs were statistically significantly increased in high dose males (~2.6x). A statistically significant increase was noted in high dose females, but the sponsor contends the values were within "the normal range of our historical values." When considered in conjunction with the histopathology findings, these are considered toxicologically significant increases.

A significant increase in cholesterol (56%) and phospholipids (43%) was found in the high dose females only. A statistically significant but not biologically significant increase was noted in the 50 mg/kg/d females. All other clinical chemistry changes were considered toxicologically insignificant.

- Urinalysis: N-Acetylglucosaminidase (NAG) increases were reported in the high dose males (3x) and females (3.2x).
- Organ Weights: Absolute (+51%) and relative (+54%) liver weights were increased in the high dose females. A 19% increase in relative liver weights was recorded for the high dose males but correlated with the lower body weights in these animals.

Cecal weights (filled and empty) were increased in all dosed males and mid and high dose females.

Relative (+17%) and absolute (+24%) kidney weights were increased in high dose females, but no histologic correlates were noted.

Thymic weights were decreased ~20% in the high dose males.

- Gross pathology: Cecae were increased in dosed animals, an expected finding with antimicrobial agents in rodents. The aforementioned alopecia in females was also noted at study termination.

- Histopathology: Increased mononuclear cell foci were reported in the liver of high dose animals (11/15 males, 13/15 females) with some single cell necrosis and/or necrotic foci. Additionally, bile duct cell clarification and mesenteric lymph node histiocytosis were noted (mid [4/15 males, 11/15 females], and high dose animals [15/15 males, 14/15 females]), consistent with a phospholipidosis (previously described).

Conclusions: The sponsor considered 50 mg/kg/d to be the NOAEL.

Histopathology Inventory for IND #

Adrenals	X
Aorta	X
Bone Marrow smear	X
Bone (femur)	X
Brain	X
Cecum	X
Colon	X
Duodenum	X
Epididymis	X
Eye	x
Gross lesions	X
Heart	X
Ileum	x

Jejunum	x
Kidneys	x
Liver	x
Lungs	x
Lymph nodes mandibular	x
Lymph nodes, mesenteric	x
Mammary Gland	x
Optic nerves	x
Ovaries	x
Pancreas	x
Parathyroid	x
Pituitary	X
Prostate	X
Salivary gland	X
Sciatic nerve	X
Seminal vesicles	X
Skeletal muscle	X
Skin	X
Spinal cord	X
Spleen	X
Sternum	X
Testes	X
Thymus	X
Thyroid	X
Tongue	X
Trachea	X
Urinary bladder	X
Uterus	X
Vagina	X

Study Title: Oral 6 Month Toxicity Study of HMR 3647 in Rats with a Four Week Recovery Period
Study No: 99/0273
Vol #, and page #: 19, page 24
Conducting laboratory and location: HMR, Frankfurt Am Main, Germany
Date of study initiation: 5/20/98
GLP compliance: Yes
QA-Report: Yes
Methods:
Dosing:

- species/strain: Hsd: Sprague Dawley SD rats at 2/cage
- #/sex/group or time point: 25 (20 as main study animals, 5 as recovery group animals)
- age: 5-6 weeks of age
- weight: ~130 gms
- dosage groups in administered units: 0 (0.5% methylcellulose), 20, 50 or 150 mg/kg/d
- route, form, volume, and infusion rate: Gavage at 10 mL/kg

Drug, lot and % purity: Analytical certificate DA-8NO364B-1 dated 5/12/98
Formulation/vehicle: 0.5% methylcellulose
Observations and times:

- Clinical signs: Twice/day during the week, once/day on weekends and holidays
- Body weights: Weekly
- Food consumption: Weekly
- Ophthalmoscopy: Prestudy, Weeks 14, 18 and 23. Recovery period animals tested Week 30.
- Hematology: After 3 months of dosing, at the end of the main study and end of recovery period
- Clinical chemistry: Same as for hematology. In addition to the usual parameters, they included phospholipids, transferrin, transferrin saturation and iron level determinations.
- Urinalysis: 10/dose/sex at 1 week prior to main study termination and all recovery animals one week prior to end of recovery period.
- Gross pathology: All animals at termination.

- Organs weighed: Adrenals, brain, epididymides, heart, kidneys, liver, lungs, ovaries, pituitary, prostate, seminal vesicles, spleen, testes, thyroids, and uterus
- Histopathology: Tissues listed below for controls (main study and recovery) and high dose (main study and recovery). Lungs, liver, gross lesions, mesenteric lymph nodes, eyes and optic nerves from low and mid dose animals.
- Toxicokinetics: In Months 1 and 5, 2 weeks prior to blood sampling from the first 18 rats/sex/dose at ~0, 1, 2, 4, 8, and 24 hours post-dosing with 3/sex/dose/timepoint. Controls were only sampled at 1 hour post-dosing. This portion of the study was performed by HMR in Cedex, France using [redacted] HPLC and fluorimetry. Limit of quantitation was [redacted]
- Other: Neurologic status: Weekly

Results:

- Clinical signs: Ptyalism was reported in all treated animals with increased incidence with increasing dose. The sponsor attributed this to the bitter taste of the test compound.
- Body weights: Females had slightly higher gains (up to ~10%) than males, but the finding was not considered toxicologically significant.
- Food consumption: Mid and high dose males ate ~8% more than females in the same groups.
- Ophthalmoscopy: 3 high dose and 1 mid dose male and female had bilateral cataracts, as did one untreated 'spare' rat. Pallor of the retina was observed in these animals. Other ophthalmic findings were considered due to injury sustained during blood sampling.
- Hematology: No significant treatment effects were reported. Interim sampling: There was a dose dependent decrease in rbc counts, especially at the high dose (-10%). In the low dose animals, a decrease of ~3% was reported. MCV was increased in mid and high dose males only.

Main study termination: High dose males had a statistically significant decrease in rbc (-6%), hematocrit and hemoglobin, with a concomitant increase in MCV (~5%) in the mid and high dose males. Mid and high dose females had decreased rbc (-9%) and hemoglobin, and high dose females had significantly decreased hematocrit with increased MCV (in all treated females but not dose dependent). The sponsor contends that the changes were only minor and were not exacerbated by 6 month dosing when compared to the 3 month dosing.

Recovery termination: MCV was still increased in the high dose males. Only 2 samples from high dose females were usable so no conclusions could be drawn. However, the rbcs were still decreased and MCV levels were increased in this limited sampling.
Leukocyte counts were increased at the interim (high dose females- +47%) and main study termination (mid dose females and both sexes in high dose) with increased neutrophils.

Differences in APTT, PT and platelets were considered incidental, but they are probably inaccurate as the retrorbital method of bleeding was used.

- Clinical chemistry: No significant treatment effects were reported by the sponsor. However, at the interim test point, increased AST (+203% in mid dose males, +156% in high dose males, +147% in high dose females), ALT (low dose +112%, +202% for mid dose males, high dose +179%, +120% for mid dose females, +150% for high dose females), and significantly increased BUN in high dose females only. Alkaline phosphatase levels were increased +65% for high dose males. All other differences from controls were sporadic and inconsistent.

Main study termination: Increased ALT (+112% low dose males, +125% mid dose males, +285% high dose males, +131% high dose females) and AST (+115% mid dose males, +205% high dose males) were reported. Alkaline phosphatase was increased +125% in high dose males, +110% mid dose females and +152% high dose females. Slight to moderate increases in liver weights were observed in males and females, respectively, correlating with the clinical chemistry increases. BUNS were increased in high dose females, but no differences from controls were noted for creatinine. These females also showed increased cholesterol and phospholipids.

Recovery termination: High dose males continued to show increased AST (+157%) and ALT (+154%). High dose females had +145% increase in AST and +166% increase in ALT when compared to controls.

- Urinalysis: No significant treatment effects were reported. However, the high dose animals had significantly increased urine volume (males: +52%, females: +300%), as did the mid dose females (+58%). NAG was significantly increased in the high dose animals (males: +154%, females: +70%). The NAG remained increased in the recovery high dose males (+37%). Proteinuria was noted in mid and high dose animals. Sporadic differences from controls were noted in some other parameters, but no obvious dose relationship was present.

- Organ Weights: Higher absolute and relative liver weights were increased in the mid dose females (+115%) and 150 mg/kg animals (+115% in males, +148% in females), as were increased relative lung weights (+14% for high dose males, +23% for high dose females).
Relative kidney weights in mid (+4%) and high dose (+12%) females were increased as were absolute weights. Relative kidney weights were increased in high dose males (+8%) but the absolute weights were comparable to controls.

Recovery termination: Relative liver weights were increased by +11% in high dose males and 10% in the high dose females. Relative kidney weights were significantly increased (+109%) in high dose males only. All other differences from controls were considered incidental with high standard deviations within groups.

- Gross pathology: Enlarged stomachs and cecae were reported in the high dose group. They were attributed to treatment, but no histologic correlates were found. Eye changes as discussed earlier were found at necropsy.

- Histopathology: The animals with bilateral cataracts had marked/severe diffuse retinal atrophy. Other sporadic animals from each group had focal/diffuse retinal atrophy without lens changes. The sponsor considered these changes to be a 'strain specific problem'. Eyes from all main study animals and 5M/6F 'spares' were subjected to external peer review.

Livers from the high dose animals showed vacuolation of the bile duct epithelium (males: 18/20, females: 20/20), consistent with the shorter term studies in rats. These findings correlated somewhat with the increased liver enzymes and liver weights. This lesion was reversible with no vacuolation found in recovery animals.

Alveolar histiocytosis was seen in the lungs of the high dose animals (19/20 males, 18/20 females) and mid dose animals (11/20 males, 17/20 females) with a clear cut dose-dependent increase in severity. Mesenteric lymph node sinuses (high dose animals: Males: 17/17, females: 18/20) also showed histiocytosis (dose-dependent increase in severity). These changes were partially reversed at the end of the recovery period. All of these changes are considered consistent with phospholipidosis, found in previous rat studies with this compound.

- Toxicokinetics: Cmax was reached within 1-2 hours after dosing. Plasma concentrations were higher in females than males at study termination with Cmax and AUC increased ~2x between the two test points. Additionally, the Cmax increased in females by 1.6 in the 50 mg/kg/d group and 2.2 in the 150 mg/kg/d group. The AUC (0-24) for females was 4.9x higher than for males at the high dose. Plasma concentrations increased more than dose proportionally, especially at the 2 highest doses.

Parameter	Day	Time	Dose		
			20 mg/kg/d	50 mg/kg/d	150 mg/kg/d
Mean Plasma Conc. (Cmax)	30	1 hr	0.033	0.144	2.02
		2 hr	0.323	0.68	5.1
		8 hr	LOQ	0.215	2.30
		24 hr	LOQ	LOQ	LOQ
	163	1 hr	0.261	0.289	1.77
		2 hr	0.279	0.92	4.18
		8 hr	LOQ	0.252	1.01
		24 hr	LOQ	LOQ	0.056
AUC (0-24 hr) mg.L.hr	30		0.92	5.0	61
	163		1.7	10	130
AUC (0-∞) mg.L.hr	30		0.67	3.8	61
	163		1.6	6.7	130

Key Study Findings: The sponsor considered the NOAEL for this study in rats to be 50 mg/kg/d. They considered neither the liver enzyme changes in the 50 mg/kg/d group, nor the renal weight changes in this group nor the increased urinary volume with increased NAG to be of toxicologic significance. The sponsor also considered the alveolar and lymph node histiocytosis and vacuolation of bile duct epithelium to be consistent with the diagnosis of phospholipidosis, demonstrated in the shorter term studies with this ketolide. While 50 mg/kg/d appears to be a NOAEL, the NOEL for this 6 month rat study is determined to be 20 mg/kg/d.

Addendum list:

Addendum 1

Histopathology Inventory for IND #

Adrenals	X
Aorta	X
Bone (femur)	X
Brain	X
Cecum	X
Colon	X
Duodenum	X
Epididymis	X
Esophagus	X
Eye	X
Gross lesions	X
Harderian gland	X
Heart	X
Ileum	X
Jejunum	X
Kidneys	x
Liver	X
Lungs	X
Lymph nodes mandibular	X
Lymph nodes, mesenteric	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: Two Week Preliminary Toxicity Study of HMR 3647 by Intravenous Route in Rats
Study No: 14704 TSR
Vol #, and page #: 22, page 52
Conducting laboratory and location:

Date of study initiation:
GLP compliance: Yes
QA- Report: Yes
Methods:
Dosing: This study was conducted to determine the doses for the 4 week study

- species/strain: Sprague-Dawley rats
- #/sex/group or time point: 6
- age: 6 weeks of age
- weight: 150- 195 gms
- dosage groups in administered units: 0, 10, 30, 60 or 90 mg/kg/d
- route, form, volume, and infusion rate: I.V. infusion over 60 minutes/day

Drug, lot: HMR 3647, batch #9
Formulation/vehicle: 0.9% NaCl with HCl (Normadose) to adjust pH.
Observations and times:

- Clinical signs: Daily
- Body weights: Once/week
- Food consumption: Once/week
- Hematology: At study termination
- Clinical chemistry: At study termination
- Urinalysis: At study termination
- Gross pathology: All animals
- Organs weighed: Adrenals, brain, heart, kidneys, liver, lungs, spleen, thymus
- Histopathology: Gross lesions, injection sites, kidneys, liver, heart and lungs only.
- Toxicokinetics: Samples were taken on Day 14

Results:

- Clinical signs: 1/6 males from the 30 mg/kg group, 2/6 from the 60 mg/kg group, and 1/6 from the 90 mg/kg group died prematurely. The sponsor attributed the deaths to the 'administration procedure (restraining tube)'.
- Body weights: No treatment-related effects were noted.
- Food consumption: No treatment-related effects were noted.
- Hematology: Increased leukocyte counts were reported for the 60 (1.5x controls) and 90 (2.2x controls) mg/kg/d groups. This was due to increased neutrophil counts (also reported for 30 mg/kg/d females).

APTT (high dose animals: -24%) was moderately decreased and fibrinogen levels were increased for all females and two top dose male groups. The sponsor attributed all of these changes to be secondary to injection site reactions. However, the samples were taken from the orbital sinus so the validity of the data is questionable.

- Clinical chemistry: No treatment-related effects were noted.
- Urinalysis: No treatment-related effects were noted.
- Organ Weights: No treatment-related effects were reported.
- Gross pathology: No treatment-related gross lesions, other than injection site reactions were reported.
- Histopathology: Injection site lesions (thrombosis of vein, inflammatory cell infiltration, collagen degradation, fibroplasia/edema in perivenous tissues) were noted in all treated groups in a dose-related fashion when compared to controls. No treatment-related systemic effects were noted. Minimal multifocal coagulative necrosis was reported sporadically in each treatment group.
- Toxicokinetics: Submitted as a separate report (97/9906/CN- Volume 23, page 6). In this report, the sponsor suggested that Cmax increased proportionally with doses of 10-90 mg/kg/d in males and 10-60 mg/kg/d in females. The Cmax in 90 mg/kg/d females was comparable to the value for the 60 mg/kg/d. Between animals variability was quite significant.

Key Study Findings: The sponsor concluded that 90 mg/kg/d was an appropriate top dose for the 4 week study.

Study title: **Changes in the Plasma Concentrations of HMR 3647 after 1 or 15 Oral Administrations of 100, 400, or 1000 mg/kg During a Preliminary Toxicology Study in the Dog**
Study No: 96/8758/CN. The in vivo portion is reported as 96/8484/TX.
Vol #, and page #: 25, pg. 276
Conducting laboratory and location: [redacted]

Date of study initiation: March-May, 1996
Methods:
Dosing:

- species/strain: beagle dogs
- #/sex/group or time point:1
- age: ~ 6 months
- weight: 6.7 kg
- dosage groups in administered units: 100, 400 or 1000 mg/kg/day
- route, form, volume, and infusion rate: Orally in capsules

Drug, lot: HMR 3647, batch 8
Observations and times:

- Toxicokinetics: Samples were taken on Days 1 and 15 at 0, 0.5, 1, 2, 4, 8 and 24 hours post-dosing.

Results: All of the animals had intermittent vomition so the actual administered/absorbed dose is uncertain.

- Toxicokinetics: For the single dose animals, the Cmax was reached between 2-4 hours (depending on vomition). No linearity was appreciated between dose and Cmax and Cmax was between [redacted] μg/mL.

After 15 doses, the Cmax and AUCs were higher than on Day 1. The Cmax was [redacted] μg/mL.

Key Study Findings: Due to the small number of animals and uncertainty of dosing, no conclusions can be drawn from this study.

Study Title: 13 Week Toxicity Study of HMR 3647 by Oral Administration (Capsules) in Beagle Dogs Followed By a 12 Week Recovery Period
Study No: 14869/TCC
Vol #, and page #: 27, page 2
Conducting laboratory and location: [redacted]
Date of study initiation:
GLP compliance: Yes
QA- Report: Yes
Methods:
Dosing:

- species/strain: Beagle dogs
- #/sex/group or time point: 4/main study groups, 2/controls
- age: Approximately 7 months old
- weight: Males: 8.2-9.7 kg; females: 7.0- 8.1 kg)
- satellite groups used for toxicokinetics or recovery: 2/control and high dose recovery groups
- dosage groups in administered units: 0 (empty capsules), 20, 50 or 150 mg/kg/d
- route, form, volume, and infusion rate: Oral

Drug, lot: 96A0179B with an expiration date of 5 Dec 1998.
Formulation/vehicle:
Observations and times:

- Clinical signs: Twice daily
- Body weights: Once pre-dosing, once on Day 1, and weekly thereafter.
- Food consumption: Estimated throughout the study
- Ophthalmoscopy: Pre-dosing and Weeks 7 and 13 at 2-4 hours post-dosing and weekly during recovery
- EKG: Pre-dosing and Weeks 5, 13, and 23 at 2-4 hours post-dosing using Standard lead II. Blood pressures were taken pre-dosing and Weeks 13 and 23 at 2-4 hours post-dosing using an electrosphygmomanometer without anesthesia.
- Hematology: Pre-dosing and Weeks 4, 12 and 24.
- Clinical chemistry: Pre-dosing and Weeks 4, 12 and 24
- Urinalysis: Pre-dosing and Weeks 12 or 13, and 24.
- Gross pathology: All animals
- Organs weighed: Adrenals, brain, epididymides, heart, kidneys, liver, lungs, ovaries, pituitary, prostate, salivary glands, spleen, testes, thymus, thyroids with parathyroids, uterus.

- Histopathology: Tissues listed below. Although electron microscopy had been planned on the eyes, the tissues were inadequately preserved for this examination so it was not performed.
- Toxicokinetics: Samples taken Weeks 4 and 13 at 0, 0.5, 1, 2, 4 and 8 hours post-dosing. Results were reported as 97/9950/CN.

Results:

- Clinical signs: One high dose male died prematurely. He had shown 'brownish vomiting' (Day 7) and increased salivation from Day 7 until he died. He also showed hypotonia and emaciation with feed consumption decreased ~70%. Ante-mortem examinations showed dehydration and secondary renal failure. Gross lesions included reddening of the GI tract, and a small thymus. Histologic examination showed renal changes, vascular dilatation in GI tract, marked histiocytosis in the mesenteric lymph nodes. The sponsor attributed all of these findings to "individual sensitivity" to the test substance and "cannot be formally attributed to the treatment."
- Slight (50 mg/kg animals) to moderate (150 mg/kg animals) vomition was seen in all treated animals during the study. Increased salivation was reported for mid and high dose groups with dose-related incidence and severity. More signs were reported during the last weeks of dosing. Although no incidence of excessive salivation was reported in the 20 mg/kg group, the sponsor stated: "Vomiting and excessive salivation were considered to be the consequence of treatment with the test substance at the dose-levels of 20, 50 and 150 mg/kg/d."

- Body weights: Gains were lower in mid and high dose animals (statistically significant) during the study but increased gains were found during the recovery period.

- Food consumption: High dose females consumed less feed (~50%) than controls during the last 4 weeks of dosing. They ate more during the recovery period.

- Ophthalmoscopy: 8/11 high dose animals showed a change in the coloration of the tapetal fundus by Week 13 of dosing. The change in coloration was due to loss of reflectivity (4/5 males, 4/6 females). Lesions were bilateral. This change was partially reversible (in most animals) as evidenced by "scattered tiny spots of normally colored tapetum". The sponsor considered these changes to be treatment-related and "specific to the tapetal species like dogs but not relevant for non-tapetal species like humans."

- Electrocardiography: Increased heart rates were reported for the 50 and 150 mg/kg/d groups during the Week 5 and 13 examinations.

Mean Heart Rates (Counts/min) for Dogs Treated with HMR 3647

Dose (mg/kg/d)	0	20	50	150
Male pretest: 112 Female pretest: 111				
Week 5 Male	98	123	136	184**
Female	107	98	129	169*
Week 13 Male	83	123	149	169**
Female	84	86	128	158*

*p<0.05; **p<0.01

The sponsor attributed the increase in heart rate to a shortening of the PQ (from 10 to 8 milliseconds in males; from 11 to 8 milliseconds in females) interval. They reported no change in QRS or QT intervals and no waveform changes in any animals. However, QT was decreased from 21 to 19 milliseconds in high dose animals at Week 13 (statistically significant in males). At the mid dose, the increased rate was not statistically significant, but all individual values were above the highest control value. At the high dose, the increased rate was statistically significant and all individual values were above the highest control value. Thus, they concluded that the heart rate changes were treatment-related. However, since the increased rate was "without clinical consequences and was not correlated to an increased heart rate or to histopathological changes in the heart, this was not considered to be a toxicological effect." No changes in diastolic or systolic pressure were reported.

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

- Clinical chemistry: ALT (males [4.7x] and females [3.9x]) and AST (males only [2x]) were increased in high dose animals during Weeks 4 and 13. The differences from concurrent controls were statistically and biologically significant. The values were "generally above the upper range of our historical control data". All

parameters were comparable in the recovery animals. As expected, gamma globulin levels were significantly decreased in the treated animals.

- Urinalysis: No significant treatment-related effects were reported.
- Organ Weights: Mean absolute and relative liver weights were increased in high dose males (22% and 41% respectively). Mean % body weight for liver was 2.74% for control males, 3.87% for high dose males; 3.01% for control males, 3.22% for high dose females. This correlated with the hepatocellular hypertrophy reported histologically. Prostatic weights were decreased in treated males but the significance is questionable in this age dog. In the recovery animals, absolute and relative liver weights were increased in the treated animals when compared to controls (2.35% of b.w. for controls vs. 2.69% for 150 mg/kg males; 1.83% of body weight for controls vs. 2.3% for 150 mg/kg females).
- Gross pathology: No treatment-related gross lesions were reported.
- Histopathology: No pathology narrative is presented. Hepatocellular hypertrophy was reported in all high dose males (slight for 1, moderate for remaining 3) and 2/4 high dose females at the end of the dosing period. Reversibility was found at the end of the recovery period. The sponsor considered this a treatment-related effect, but considered it a normal response to an increased demand in liver function.
- Toxicokinetics:

		Males			Females		
Dose (mg/kg/d)		**20**	**50**	**150**	**20**	**50**	**150**
Cmax (mg/L)	Day 26	3.36	6.8	18.3	2.76	7.34	15.4
	Day 89	2.70	7.56	14.5	2.71	7.48	12.6
Tmax (Min-max hours)	Day 26						
	Day 89						
AUC $_{0\text{-}24\ hr}$ (mg/L.hr)	Day 26	17.1	60.4	240	17.0	54.8	179
	Day 89	16.9	65.0	205	19.9	57.9	183
C_{24hr} (mg/L)	Day 26	LOQ	0.112	4.3	LOQ	0.108	2.2
	Day 89	LOQ	0.082	4.44	LOQ	0.083	4.49

The variability between animals was moderate to high. There was no apparent gender effect. AUC and Cmax increased with duration of dosing and deviated from proportionality (lower than increase for Cmax, higher than increase for AUC). The results were similar in the 30 day dog study #96/9158/CN.

Key Study Findings: The sponsor considered the NOAEL for this 13 week study in the dog to be 50 mg/kg/d on the basis of the clinical signs, weight gain reduction, clinical chemistry findings and ophthalmoscopic changes in the high dose animals.

Addendum 1

Histopathology Inventory for IND #

Adrenals	X
Aorta	X
Bone (femur) with joint	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
Jejunum	X
Kidneys	X
Liver	X
Lungs	X
Lymph nodes mandibular	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
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: Toxicokinetics of HMR 3647 for Study 14688 TSC: Four Week Toxicity Study by I.V. Route in the Beagle Dog

Study No: 97/9903/CN. The *in vivo* portion of this study was reported as 14688TSC.

Vol #, and page #: 30, pg. 306

Conducting laboratory and location: HMR, Cedex, France

Date of study initiation: 11/13/96

GLP compliance: Yes

QA- Report: Yes

Methods:

Dosing:

- species/strain: Beagle dogs
- #/sex/group or time point: 3
- age: ~7 months of age
- weight: 7.0- 8.9 kg
- dosage groups in administered units: 0, 10, 30 or 90 mg/kg/d for 30 days
- route, form, volume, and infusion rate: I.V. over one hour

Drug, lot: Batch 9

Formulation/vehicle: 0.9% NaCl

Observations and times:

- Toxicokinetics: Samples were drawn on Day 30 just before the end of the infusion and 24 hours after the last infusion.

Results:

- Toxicokinetics

Dose (mg/kg/d)	Sex	10	30	90
Parameter				
Cmax Mg/L	Male Female	2.59±0.23 2.41±0.41	10.03±0.79 7.63±0.04	23.69±2.0 26.22±1.48
C_{24hr} Mg/L	Male Female	LOQ LOQ	LOQ LOQ	0.54±0.08 0.51±0.13

Key Study Findings: Variability between animals was relatively small, especially considering other PK values in previously reviewed studies. Cmax increased proportionally with dose.

Study title: **Pharmacokinetics of HMR 3647 in the Young Dog After One or Ten Oral Administrations of 100 mg/kg**
Study No: 98/10420/CN. The in vivo portion is reported as the bone toxicity study #95/7829/TX
Vol #, and page #: 31, page 78
Conducting laboratory and location:
Date of study initiation:
GLP compliance: Yes.
QA- Report: Yes
Methods:
Dosing:

- species/strain: Beagle dogs
- #/sex/group or time point: 3/sex for the repeat dose portion, 2/sex for the single dose portion
- age: For the repeat dose study, 6.5-8 weeks of age. For the single dose study, 7.5 weeks of age
- weight: Mean: 2.1 kg for the repeat dose study; 1.3 kg for the single dose study
- dosage groups in administered units: 100 mg/kg
- route, form, volume, and infusion rate: By gavage at 2 mL/kg

Drug, lot: 5A0131B, batch 4
Formulation/vehicle: 0.5% methylcellulose

- Toxicokinetics: Samples were taken at 0.5, 1, 2, 4, 6 and 24 hours after the 1st or 10th administrations.

Results:

- Toxicokinetics: The single dose portion: 2 females vomited after dosing so the actual exposures are questionable. Three of four single dose animals reached Cmax (overall mean: 9.2 μg/mL) at 2 hrs. while the fourth reached Cmax (— μg/mL) at 30 minutes. The elimination t1/2 was approximately 4 hours. The AUC was a mean of 84.0 μg.mL/hr. Significant interanimal variations were reported.
- The repeated dose portion: Cmax (overall mean: 7.6 μg/mL) was reached at 2 hours. The elimination t1/2 was approximately 4 hours. The AUC was a mean of 80.7 μg.mL/hr. Significant interanimal variations were reported. No drug accumulation was demonstrated.

Key Study Findings: Cmax and AUC were comparable whether the animals received 1 or 10 doses of the test compound.

Study Title: **A Preliminary 2 Week Repeated Dose Toxicity Study of HMR 3647 Administered Orally to Young Beagle Dogs**
Study No: — /8-82
Vol #, and page #: 31, page 108
Conducting laboratory and location: —
Date of study initiation: 11/20/98
GLP compliance: No. Many of the tables and narrative are presented in the original Japanese without translation.
Methods:
Dosing:

- species/strain: Beagle dogs
- #/sex/group or time point: 1/sex/group
- age: 3 weeks at initiation of dosing
- weight: 0.74-1.0 kg on receipt
- dosage groups in administered units: 0, 150, or 300 mg/kg/d for 14 days
- route, form, volume, and infusion rate: Orally as capsules

Drug, lot: Batch 22
Observations and times:

- Clinical signs: Three times/day
- Body weights: Twice/week
- Food consumption: Appetite was assessed daily but pups were left with dams during the nights
- Hematology: Pre-dosing and at study termination
- Clinical chemistry: Pre-dosing and at study termination
- Gross pathology: All animals
- Organs weighed: None