group. Other species (monkeys and rats) have not demonstrated histopathological changes in the eye following moxifloxacin dosing. Neither of these species has a tapetum in the eye, unlike the dog. This may be an explanation for the species specificity of the retinal changes. Humans are also a non-tapetal species.

Accelerated Bioassay in the Rat of BAY 12-8039 Quinolone (QL) in Target Organs of Human Carcinogenesis (AHF Study R-1806) (Bayer Report No. R 7239)

[redacted]

Report dated: 8/14/98, [redacted]

Vol. 35, pp. 1-581

Animals: Male and female Wistar Rats (females were used for assessment of mammary tumors, and males were used for all other targets), about 8 weeks old at the initiation of treatment, housed 2/cage, 18-28 rats per group

Diet: [redacted] diet [redacted] and tap water were available *ad libitum*.

Treatment Groups and Study Conduct: Moxifloxacin (Batch Nos. 960704A, 521725C, 522381D, 970423A) was suspended in 0.5% carboxymethyl cellulose and administered orally via gavage at a dose of 500 mg/kg (based on the HCl salt; dose would be 459 mg/kg based on free base). Depending upon the treatment group, it was given 7 days a week for 38 weeks, for 13 weeks at the beginning of the treatment period, or for the last 24 weeks of the treatment period. Other test substances used in the various treatment groups were: diethylnitrosamine (DEN- liver tumor initiator), dissolved in 0.9% saline, given orally via gavage once a week for the first 9 weeks of treatment at 10 µmoles/kg; ethylnitrosourea (ENU-bone marrow initiator), dissolved in sterile 0.9% saline, given IV at 20 mg/kg once a week for the first 9 weeks of treatment, N-butyl-N-(4-hydroxybutyl)nitrosamine (BBN-urinary bladder tumor initiator), given daily in the drinking water at 0.02% for the first 9 weeks of the study; N-nitrosodimethylamine (NDA- lung tumor initiator), dissolved in 0.9% saline, given orally via gavage at 7.5 mg/kg once a week for the first 9 weeks of treatment, 7,12-dimethylbenz[a]-anthracene (DMBA- mammary tumor initiator), dissolved in olive oil, a single dose given orally via gavage on day 1; methylnitrosourea (MNU- nonglandular stomach tumor initiator), dissolved in 0.9% saline, given orally via gavage at 4.5 mg/kg ~~once a~~ week for the first 9 weeks of treatment; phenobarbital (PB- liver tumor promoter), given in the diet at 500 ppm for the last 24 weeks of the study; azathioprine (AZ- bone marrow tumor promoter), dissolved in 0.9% saline, given orally via gavage 5 days/week for the last 24 weeks of treatment; nitrilotriacetic acid trisodium salt monohydrate (NTA-urinary bladder tumor promoter), given in the diet at 10,000 ppm for the last 24 weeks of the study; butylated hydroxytoluene (BHT-lung tumor promoter), given in the diet at 10,000 ppm for the last 24 weeks of the study; butylated hydroxyanisole (BHA- nonglandular stomach tumor promoter), given in the diet at 6,000 ppm for the last 24 weeks of the study; and diethylstilbestrol

(DES- mammary gland tumor promoter), dissolved in sterile 0.9% saline, given subcutaneously once a week at a dose of 0.2 mg/kg for the last 24 weeks of treatment.

Treatment lasted for a total of 38 weeks (unless interim sacrifices of 4 animals/group were performed). The study was performed in 4 phases so that sacrifices could be reasonably managed. The groups for each phase of the study were as follows:

Phase 1- Males:

Target Tissues: Liver and Kidney

1. Moxi (first 13 weeks) + PB (last 24 weeks) (interim sacrifices at 14 and 26 weeks)
2. DEN (first 9 weeks) + Moxi (last 24 weeks) (interim sacrifices at 14 and 26 weeks)
3. PB (last 24 weeks) only (interim sacrifices at 14 and 26 weeks)
4. DEN (first 9 weeks) only (interim sacrifice at 26 weeks)
5. DEN (first 9 weeks) + PB (last 24 weeks) (interim sacrifice at 26 weeks)

Target Tissue: Lung

6. Moxi (first 13 weeks) + BHT (last 24 weeks) (interim sacrifice at 26 weeks)
7. NDA (first 9 weeks) + Moxi (last 24 weeks) (interim sacrifice at 26 weeks)
8. BHT (last 24 weeks) only (interim sacrifice at 26 weeks)
9. NDA (first 9 weeks) only (interim sacrifice at 26 weeks)
10. NDA (first 9 weeks) + BHT (last 24 weeks) (interim sacrifice at 26 weeks)

11. Vehicle Control (interim sacrifices at 14 and 26 weeks)
12. Moxi only for 38 weeks (interim sacrifices at 14 and 26 weeks)

Phase 2- Females:

Target Tissue: Mammary Gland

13. Moxi (first 13 weeks) + DES (last 24 weeks) (interim sacrifices at 14 and 26 weeks)
14. DMBA (single dose on day 1) + Moxi (last 24 weeks) (interim sacrifices at 14 and 26 weeks)
15. DES (last 24 weeks) only (interim sacrifices at 14 and 26 weeks)
16. DMBA (single dose on day 1) only (interim sacrifices at 14 and 26 weeks)
17. DMBA (single dose on day 1) + DES (last 24 weeks) (interim sacrifices at 14 and 26 weeks)

18. Vehicle Control (interim sacrifices at 14 and 26 weeks)
19. Moxi only for 38 weeks (interim sacrifices at 14 and 26 weeks)

Phase 3- Males:

Target Tissues: Kidneys and Urinary Bladder

20. Moxi (first 13 weeks) + NTA (last 24 weeks) (interim sacrifice at 26 weeks)
21. NTA (last 24 weeks) only (interim sacrifice at 26 weeks)
22. DEN (first 9 weeks) + NTA (last 24 weeks) (interim sacrifice at 26 weeks)
23. BBN (first 9 weeks) + Moxi (last 24 weeks) (interim sacrifice at 26 weeks)
24. BBN (first 9 weeks) only (interim sacrifice at 26 weeks)
25. BBN (first 9 weeks) + NTA (last 24 weeks) (interim sacrifice at 26 weeks)

Target Tissue: Stomach

26. Moxi (first 13 weeks) + BHA (last 24 weeks) (interim sacrifice at 26 weeks)
27. MNU (first 9 weeks) + Moxi (last 24 weeks) (interim sacrifice at 26 weeks)
28. BHA (last 24 weeks) only (interim sacrifice at 26 weeks)
29. MNU (first 9 weeks) only (interim sacrifice at 26 weeks)
30. MNU (first 9 weeks) + BHA (last 24 weeks) (interim sacrifice at 26 weeks)

Phase 4- Males:

Target Tissue: Bone Marrow

31. Moxi (first 13 weeks) + AZ (last 24 weeks) (interim sacrifices at 14 and 26 weeks)
32. ENU (first 9 weeks) + Moxi (last 24 weeks) (interim sacrifices at 14 and 26 weeks)
33. AZ (last 24 weeks) alone (interim sacrifices at 14 and 26 weeks)
34. ENU (first 9 weeks) alone (interim sacrifices at 14 and 26 weeks)
35. ENU (first 9 weeks) + AZ (last 24 weeks) (interim sacrifices at 14 and 26 weeks)

36. Vehicle Control (interim sacrifices at 14 and 26 weeks)

Cageside observations were performed once daily. Body weights were measured and animals palpated for tumors weekly for the first 13 weeks of the study and every 2 weeks for the remainder.

A satellite group of rats was given moxifloxacin as above for 98 days. Blood was collected for toxicokinetic analysis of plasma immediately prior to the final dose of drug, and 1.5, 3, 5, 8, and 24 hours after the final dose was given.

All animals found dead during the study underwent gross necropsy. At scheduled sacrifices, the liver, kidneys and spleens of the rats were weighed. Target organs (liver, kidneys, lungs, mammary glands, urinary bladder, forestomach, bone marrow) and gross lesions were fixed for at least 24 hours in neutral buffered formalin before further processing. All of the target organ tissues listed above were microscopically examined in the control rats, other target tissues were examined as per the experimental scheme described above. Gross lesions were examined

microscopically. The following tissues were preserved for all rats, but were not necessarily subjected to histopathologic examination: pituitary, thyroids, parathyroids, kidneys, lungs, liver, thymus, spleen, urinary bladder, bone marrow (from tail bone and femur), testes, mammary glands, skin and subcutaneous tissue, brain, and stomach.

Results: Mortality was high in the ENU groups (12/26 rats). Most of these rats died due to tumor burden. This left 6 rats per ENU group for the 38 week sacrifice after 4 rats per group were sacrificed at weeks 14 and 26. Other groups lost 0-5 rats over the course of the study. None of the vehicle control rats died prematurely. In the case of the animals treated with moxifloxacin only, most unscheduled deaths were due to gavage accidents. Some rats in the other groups died due to tumor burden. This left 18 rats in the moxifloxacin only group 12 and 8-14 rats in the remaining groups for the 38 week sacrifice.

Reductions in mean body weight gain and mean terminal body weights were observed for the rats treated with BHT, DES, NTA, and ENU, as well as the rats treated with NDA plus moxifloxacin.

In general, positive control initiators and promoter combinations induced tumors in the expected target organs. Moxifloxacin treatment alone was not associated with the development of neoplasms at greater rates than historical controls. The incidence of stomach tumors in rats treated with MNU and BHA was 4/18, as was the incidence in rats treated with MNU alone. The incidence in rats treated with MNU followed by moxifloxacin was 7/18, but this was not statistically greater than the other 2 groups or historical control rats treated with MNU. BHA did not appear to act as a promoter in this arm of the study.

PB did not act as a potent promoter in the liver following initiation with DEN, nor was DEN a particular potent initiator in this study. When rats were treated with DEN alone, 1/18 developed liver neoplasms. When PB followed DEN, 2/18 rats developed liver neoplasms and 1/18 developed kidney tumors. When moxifloxacin was given before PB, 1/18 rats developed a liver tumor and when moxifloxacin was given following DEN, 1/22 developed liver tumors and 1/22 had a mammary gland tumor.

NDA and BHT, when administered together, did not cause any tumors in their target tissue, lung. When NDA was given alone, 1/18 rats had a lung tumor. Moxifloxacin, given with either of these chemicals, was not associated with any neoplasms.

DES did not act as a promoter of mammary tumors following initiation with DMBA. When female rats were treated with DMBA alone, 4/22 developed mammary tumors, but only 1/22 developed such tumors following treatment with DMBA + DES and 3/22 developed mammary tumors when moxifloxacin was administered following DMBA.

In the rats treated with the initiator BBN, urinary bladder tumors were found in 100% (18/18) treated with both BBN and NTA (all carcinomas). Of the rats treated with BBN alone, 15/18 rats had tumors, with 14/18 carcinomas. Twelve of 18 rats treated with BBN and moxifloxacin had tumors, with 11/18 carcinomas. This was a significant reduction compared to the BBN + NTA group. Additionally, the tumors in the BBN + moxifloxacin group were significantly smaller (0.44 ± 0.21 cm) than those in the BBN + NTA group (1.92 ± 1.69 cm).

The investigators found that the ENU-treated rats had tumors at many target sites other than bone marrow, including the skin; large intestine, auditory sebaceous glands, small intestine, and mammary glands. ENU-induced tumors of the large intestine occurred significantly later in

the rats treated with moxifloxacin + ENU (32.0 ± 2.2 weeks) than ENU alone (28.9 ± 1.5 weeks). Combination of ENU with AZ did not increase tumor incidence.

Under the conditions of this study, moxifloxacin appeared to be neither a tumor initiator nor a tumor promoter at specific target sites (liver, kidneys, lungs, mammary glands, urinary bladder, forestomach, bone marrow) when administered to rats orally at a dose of 459 mg/kg. It should be noted, however, that most of the promoters used in this study did not appear to induce much greater incidences of tumors in target organs as did initiators alone, calling into question the sensitivity of this assay in identifying tumor promoters.

BAY 12-8039: Plasma Concentrations in Wistar Rats after Oral Administration in a Short Term Carcinogenicity Study (Bayer Report No. PH 27338; Study No. T 7061343)

C. Kohlsdorfer, H. Enzmann (Bayer, Wuppertal, Germany)

Report dated: 3/26/98

Vol. 48, pp. 232-268

Summary: This report contains the results of the toxicokinetic study performed as part of the "accelerated bioassay" in rats, above. In a satellite groups of male and female rats (3-4 female animals and 5-7 males per time point), moxifloxacin was administered daily (459 mg/kg) for 14 weeks and blood samples were drawn prior to the last dose of drug and 1.5, 3, 5, 8, and 24 hours after the final dose was administered. Plasma concentrations and AUCs of moxifloxacin were higher in male rats than in females, and Cmax was achieved earlier in the males.

Mean Plasma Concentrations (µg/ml) and Toxicokinetic Parameters in Male and Female Wistar Rats Following 14 Weeks of Daily Dosing with Moxifloxacin (459 mg/kg)

Time (hr)	Males	Females
0	1.15	0.016
1.5	8.08	2.10
3	5.78	2.48
5	5.66	1.19
8	4.49	0.302
24	1.70	0.032
AUC_{0-24} (µg·hr/ml)	89.8	12.4
Cmax (µg/ml)	8.08	2.48
Tmax (hr)	1.50	3.00

Reproduction Toxicity Studies:

BAY 12-8039: Plasma Concentrations in Pregnant Wistar Rats and Tissue Concentrations in Fetuses After Oral Administration in an Embryotoxicity Study (Bayer Report No. PH 25756; Study No. T 1054282)

C. Kohlsdorfer (Bayer, Wuppertal, Germany)

Report dated: 12/16/96

Vol. 37, pp. 354-385

Summary: This report contains the results of the toxicokinetic study performed as part of Study No. 617253. Samples were collected at and shipped to Bayer for analysis. Moxifloxacin (20, 100, 500 mg/kg suspended in 0.5%) was administered orally to pregnant female rats on days 6-17 of gestation. Blood samples were collected 1.5, 3, 7, and 24 hours after dosing on day 16 from 4 dams per dose group. On day 17, blood was collected from the dams again 1.5 hours after dosing, then the animals were sacrificed immediately and their fetuses harvested so that the amount of moxifloxacin in the fetuses could be measured. Fetuses were pooled by litter for analysis. A validated was used to quantitate moxifloxacin in plasma (detection limit 5 ng/ml) and tissues (detection limit 30 ng/ml).

The highest plasma levels were recorded at the 1.5 hr time point, but it is difficult to know if this is a true Cmax because it was the first sample drawn. Blood levels for moxifloxacin 1.5 hours after dosing were 0.071, 0.456, and 2.58 µg/ml for 20, 100, and 500 mg/kg doses. AUC for each dose was estimated from time 0 until the last blood sample with detectable moxifloxacin (7 hours for the 2 lower doses and 24 hours for 500 mg/kg). The AUCs for 20, 100, and 500 mg/kg were 0.176, 1.24, 11.6 µg·hr/ml, respectively. The half life of moxifloxacin in the dams was 4-5 hours.

The maternal plasma concentrations measured 1.5 hours after dosing on days 16 and 17 were similar. The fetal concentrations of moxifloxacin were 0.135, 1.07, and 5.37 µg/g for the 20, 100, and 500 mg/kg dose groups.

Study of Pre- and Post-Natal Development in Rats After Oral Administration (Bayer Report No. PH 27379

B. Stahl (Bayer, Wuppertal, Germany)

Report dated: 3/16/98

Vol. 38, pp. 1-541

Animals: Wistar rats (Hsd Cpb:WU); 11-16 weeks old at the time of mating (females, 180-237 g; males, >300 g); a pair of females was housed with one male until insemination was confirmed by the presence of a copulatory plug; females were randomly assigned to dose groups of 28 rats

Diet: [redacted] and tap water were supplied *ad libitum*.

Drug Dose and Route of Administration: Moxifloxacin (Batch No. 960704A) was suspended in 0.5% [redacted] and administered via oral gavage at dose levels of 0 (vehicle control), 20, 100, and 500 mg/kg once daily from day 6 or pregnancy until the end of lactation. The dose volume was 10 ml/kg of body weight. The doses were selected based on the results of a previous fertility and early embryonic development study and a developmental toxicity study that was conducted in rats using the same dose levels.

Conduct and Length of Study: Pregnant dams were observed daily for mortality and clinical signs throughout the study period. Body weight was measured daily throughout the study. Food consumption was recorded for days 0-6, 6-11, 11-16, and 16-20 of gestation and from days 0-7 and 7-14 of lactation. Dams were watched carefully as the delivery time approached so that the length of gestation, length and difficulty of delivery, viability/external normality of newborns and nursing behavior could be recorded. However, as the dams had their litters at night, little information regarding delivery was available. Live offspring were sexed and weighed. Litters were culled to 8 pups (with sex distribution as homogenous as possible) 4 days after birth. Litters containing fewer than 8 pups were not adjusted. Dams with no remaining viable pups were sacrificed and their uteri examined for implantation sites. Pups were weighed on lactation days 4, 7, 14, and 21. The times of pinna detachment, eye opening, fur growth, incisor eruption and development of normal walking were noted. The time of development of normal reflexes (surface righting, negative geotaxis, pupillary reflex) was tested and hearing was tested by observing Preyer's reflex in response to tones at 6.7 kHz from an electric generator. One male and one female pup were randomly selected from each litter for motor activity testing (in a red-lit room using an automatic counter) on days 22 and 23 postpartum. One male and one female pup from each litter were randomly selected for a learning and memory test (a water M-maze) during the 5th and 6th weeks postpartum. The dams were sacrificed at the end of lactation and examined grossly; the number of implantation sites was determined. One pup per gender from each litter was randomly chosen to assess reproductive performance, including dates of vaginal opening or testes descent. The remaining F_1 pups were sacrificed and a gross necropsy was performed.

For reproductive competence testing, at about 14 weeks of age, the F_1 rats were paired with a mate from the same test group, but not the same litter. If mating failed to occur within 8 days, pairs were changed and mating was allowed to continue for 4 days more. F_1 dams were allowed to deliver their pups, which were observed grossly after birth, then sacrificed.

Results: One rat from the 20 mg/kg dose group died during the study, but the investigators did not believe ~~that the~~ death was drug-related. Six animals from the 500 mg/kg group died- four between days 9-22 of gestation and the others on days 1 and 9 (this rat sacrificed moribund) of lactation. The deaths that occurred during gestation appeared to have been drug-related as the animals lost weight prior to their deaths and the intestines and/or stomachs of these rats were red, a gross finding observed in other rats which died during studies with moxifloxacin. The two other 2 deaths did not appear drug related. The death on lactation day 1 appeared to have been due to a dosing accident (sudden death after dosing with fluid in thoracic cavity) and the rat that was moribund sacrificed on lactation day 9 had hydrocephalus internus.

Salivation was observed on a few occasions in 3 rats from the 100 mg/kg group and in 10 rats at 500 mg/kg. Reddish discoloration of saliva was seen in one of the rats in the high dose group. Coprophagia was occasionally observed in 3 rats at 20 mg/kg, 8 at 100 mg/kg and 10 at 500 mg/kg. The frequency, as well as the incidence, increased with dose. Soft feces was seen in several animals from each dose group, likely secondary to the antibacterial effects of the drug. None of these signs were seen in the control group.

Mean food consumption was slightly, but significantly reduced compared to controls during days 6-11 of gestation in the 100 and 500 mg/kg rats. The average daily food consumption during this period for controls was 19.8 g, and for the mid and high dose rats it was 18.3 g and 17.0 g, respectively. Food consumption was also slightly reduced in the high dose group compared to controls during days 16-20 of gestation (25.0 g vs. 22.8 g). Mean body weight gain was significantly lower in the 500 mg/kg rats than controls during days 6-20 of gestation (91.7 g vs. 76.3 g). The high dose dams gained significantly more weight than controls during the lactation period, however. This compensatory weight gain occurred despite the continuation of moxifloxacin dosing. Necropsy of the dams revealed no drug-related findings with the exception of the red discoloration of the GI tract in the females from the 500 mg/kg group that were found dead.

The length of gestation was slightly, but significantly increased in the 500 mg/kg dams compared to controls (22.64 days vs. 23.10 days). The length of gestation in the 500 mg/kg dams was slightly greater than historical controls. The report contained a value referred to as "prenatal loss" which was described as the difference between the number of implantation sites in a dam and the number of pups born. The mean prenatal loss was 0.73 ± 1.12 for controls, 1.38 ± 1.44 for 20 mg/kg, 0.83 ± 1.13 for 100 mg/kg, and 1.71 ± 2.45 for 500 mg/kg. The investigators did not consider the increase at 20 mg/kg to be related to moxifloxacin because it was not dose-related and was within the historical control range (the reviewer agrees). The investigators believed that the increased prenatal loss at 500 mg/kg could be related to drug. The reviewer calculated postimplantation loss for this compound and got similar results (control, $9.5 \pm 21.2\%$, 20 mg/kg, $10.7 \pm 11.1\%$, 100 mg/kg, $6.6 \pm 8.2\%$, and 500 mg/kg, $12.8 \pm 17.2\%$). As the investigators stated in the report, it is difficult to exclude the possibility that the slight increase in prenatal loss (or postimplantation loss) observed at 500 mg/kg is related to moxifloxacin- the effect seemed to vary somewhat among the dams in this dose group.

No drug-related external malformations were observed in the pups at birth and no drug-related gross internal malformations were observed at necropsy at the end of lactation. Mean pup birth weights were significantly less than control at 500 mg/kg (6.18 g vs. 5.84 g), but by day 21 of lactation, there was no longer a difference in mean body weight between these groups. Neonatal pup mortality (birth to day 4 of lactation) was increased at 500 mg/kg compared to controls. Mean pup survival at birth was $99.7 \pm 1.5\%$ in controls and $94.6 \pm 11.4\%$ at 500 mg/kg. At day 4 of lactation, pup survival was $98.0 \pm 3.8\%$ in controls and $82.6 \pm 22.8\%$ at 500 mg/kg. Survival was similar among the dose groups for the remainder of the lactation period (94-98% for all).

Moxifloxacin did not appear to affect the time to acquisition of developmental landmarks, the development of normal reflexes, or hearing. Learning and memory (tested using a water M maze) was not affected by moxifloxacin treatment. Sporadic differences in some activity parameters compared to controls (e.g., slightly increased horizontal motor activity with slightly decreased vertical activity) did not appear to be drug-related.

No impairment of fertility was observed in the F_1 generation. No differences in the mean number of implantation sites or live pups per litter were observed. Pup weights were similar among the treatment groups and no drug-related increases in gross malformations were seen.

Mortality was observed during gestation in the F_0 dams dosed with 500 mg/kg/day of moxifloxacin. Coprophagia was seen occasionally in all moxifloxacin groups and salivation was observed in some dams at 100 and 500 mg/kg. Effects suggestive of fetotoxicity (slight increase in postimplantation loss, decreased pup birth weight, and increased neonatal mortality) were seen at 500 mg/kg. However, even at 500 mg/kg, there appeared to be no adverse effect on the acquisition of developmental landmarks or the development of hearing and normal reflexes. Learning, memory, and activity level also appeared normal in the offspring of dams dosed with up to 500 mg/kg of moxifloxacin. There was no impairment of fertility in the F_1 generation at doses up to 500 mg/kg.

BAY 12-8039: Development Toxicity Study Following the Intravenous Administration in Rabbits (Bayer Report No. PH 27071; Study No. T8060903)

B. Holzum (Bayer, Wuppertal, Germany)

Report dated: 10/17/97 [redacted]

Vol. 39, pp. 1-575

Animals: CHBB:HM Himalayan rabbits; females were 2.1-3.4 kg and males were "mature" at the time of mating; females were mated 1:1 with males under observation and pregnancy was assumed after copulation was witnessed; 20 females per treatment group for reproduction study and 12 per group for toxicokinetics (results reported below)

Diet: [redacted] Rabbit Diet K-Z and tap water were provided *ad libitum*.

Drug Dose and Route of Administration: Moxifloxacin (Batch No. 960704A) was dissolved in 5% glucose (pH 4.4) and administered intravenously at dose levels of 0 (vehicle control), 2, 6.5, and 20 mg/kg. The dose volume was 1.5 ml/kg of body weight. Drug was administered to the presumed pregnant females from days 6-20 of gestation. The doses was chosen based upon the results of a pilot study in pregnant rabbits. In that experiment, mortality occurred at 50 mg/kg and food intake was severely decreased at 25 mg/kg and slightly decreased at 16 mg/kg.

Conduct and Length of Study: Pregnant does were observed twice daily for mortality and clinical signs. Body weight was measured daily. Food consumption was recorded for the following intervals: days 0-6, 6-11, 11-16, 16-21, 21-24 and 24-29. On day 29 of gestation, dams were sacrificed and their fetuses delivered by Caesarian section. Dams were examined by gross necropsy after sacrifice (or following abortion or unscheduled death). The fetuses were examined for internal soft tissue abnormalities using fresh dissection technique (modification of staples). The fetal remains were fixed, stained, and examined for skeletal abnormalities.

Results: One female in the 2 mg/kg group died on day 8 of gestation, but the death of this animal was attributed to stress from the injection technique (which involved restraint of the rabbits) rather than toxicity of moxifloxacin. Two other does, one each from the 6.5 and 20 mg/kg groups died on day 25 and 27 or gestation, respectively. Both of these rabbits were hypoactive and had dramatic decreases in food and water intakes with body weight loss prior to their deaths. Abortion occurred between days 20-29 in 1 animal from the 6.5 mg/kg group and 5 in the 20 mg/kg group. These animals had large decreases in food and water intake with body weight loss prior to abortion. Gross necropsy revealed cecal enlargement, gall bladder enlargement, and/or fatty change in liver in several of the does that died or aborted.

Mean food intake was reduced compared to control in all treatment groups, but the difference reached statistical significance for the 6.5 mg/kg group only during days 6-11. In the 20 mg/kg group, the decrease was greatest and was statistically significant during the intervals days 6-11, 11-16, and 16-21. Even the does in the 20 mg/kg group that did not abort their litters had reduced food consumption compared to control, though those which aborted had the most dramatic reductions in food intake. The incidence of reduced feces in the drug-treated dams in the 6.5 and 20 mg/kg dose groups was correlated with reduced food consumption. Reduced body weight was also associated with decreased food consumption. The does given 20 mg/kg of moxifloxacin that had viable litters had decreased body weight compared to controls; those that aborted had more severe body weight losses. Decreased water intake was associated with discolored urine, reduced urine volume, and mineralized deposits in the renal tubules seen in some 20 mg/kg does at necropsy.

Of the dams which had viable litters at the end of the study, there was no difference in litter size between controls and does given moxifloxacin at doses up to 20 mg/kg. Mean placental (4.37 vs. 3.67 g) and fetal weights (37.83 vs. 29.75) were significantly reduced in the 20 mg/kg group compared to controls, however. Examination of the placentas revealed an increased incidence in "course grained" placenta at the 6.5 and 20 mg/kg doses.

The overall incidence in fetal malformations (including external, visceral and skeletal) was increased in the 20 mg/kg group (8 fetuses in 8 litters out of 13 viable litters) compared to concurrent (2 fetuses in 2 litters out of 18 viable litters) or historical controls. The incidence of the separate malformations did not exceed historical controls, so it is difficult to attribute the malformations observed in the 20 mg/kg group to moxifloxacin. In particular, malposition of the forelimbs (ventral flexure at the wrist) is the most common spontaneous malformation in this rabbit strain in the historical control database. The investigators stated that the total number of rib and vertebral malformations in the 20 mg/kg group was slightly increased compared to historical controls.

Malformations Observed in Rabbit Fetuses After Moxifloxacin Treatment

Malformation	Concurrent Control	2 mg/kg	6.5 mg/kg	20 mg/kg
Malposition of Forelimb(s)		4 (3)	3 (3)	2 (2)
Cleft Palate				1
Missing Parts of Skull Bones	1			
Missing Kidney and Ureter	1			
Cardiac Septal Defect		2 (1)		1

Bifurcation of Ribs				1
Fusion/Bifurcation/Missing Ribs with Malformation of Vertebral Bodies		1		1
Supernumerary Lumbar Vertebra			1	
Missing Lumbar Vertebra				2 (2)

*Number of litters affected in parentheses

The visceral deviation "distinct liver lobulation" was observed more frequently in the 20 mg/kg fetuses (16 fetuses in 4 litters out of 13 total litters) than controls (1 fetus), but it is not an uncommon deviation in the historical database for this strain of rabbit. Retarded skeletal ossification was observed in the skull, phalanges and vertebrae (caudal and cervical) of the 20 mg/kg fetuses.

Signs of fetotoxicity (reduced placental and fetal weights, retarded ossification) were observed at 20 mg/kg. There was also a slight increase in the total number of malformations at 20 mg/kg and of combined rib and vertebral malformations. Maternal toxicity (including mortality) was observed at 6.5 and 20 mg/kg. Dose related increases in abortion (secondary to reduced food and water consumption and body weight loss in the does) were also observed at these levels of moxifloxacin.

BAY 12-8039: Additional Developmental Toxicity Study in Rabbits After Intravenous Administration (Bayer Report No. PH 26904; Study No. T6061333)

B. Holzum (Bayer, Wuppertal, Germany)

Report dated: 10/14/97, [redacted]

Vol. 40, pp. 1-386

Summary: The rabbit study above was repeated (same animals, batch of drug, and experimental conditions) using doses of 0 and 0.5 mg/kg/day of moxifloxacin so that a NOEL for toxicity in the does could be determined definitively. No signs of maternal or fetal toxicity were observed in this study. One rabbit in the 0.5 mg/kg group aborted on day 24 of gestation, but this was considered unrelated to treatment as the doe showed no signs of toxicity. A second doe in this group had total reabsorption of its litter. Both abortion and total litter resorption have been observed in historical controls. Overall, 18/20 does in the 0.5 mg/kg moxifloxacin group had viable litters and this is within the historical control rate. Food and water consumption and body weights of the 0.5 mg/kg does were not different from control and necropsy did not reveal any gross drug-related findings. Signs of fetotoxicity (e.g., decreased fetal or placental weights, reduced ossification, decreased fetal survival) were not seen and drug-related malformations or variations were not observed.

BAY 12-8039: Plasma Concentrations in Pregnant Rabbits After Intravenous Administration in Two Embryotoxicity Studies (Bayer Report No. PH 27221)

C. Kohlsdorfer, B. Holzum (Bayer, Wuppertal, Germany)

Report dated: 2/10/98

Vol. 40, pp. 387-437

Summary: This is the toxicokinetics report for the two intravenous rabbit developmental studies above (T 8060903 and T 6061333). Blood samples were drawn from the satellite rabbits assigned for toxicokinetics on days 6 and 19 of gestation (days 1 and 15 of moxifloxacin administration) 0.25, 1, 4, 7, and 24 hours after drug was given and 0.25 hours after the day 20 dose immediately before sacrifice. The fetuses in each litter were pooled for determination of moxifloxacin. A validated [redacted] was used to quantitate moxifloxacin in plasma (detection limit 5 ng/ml) and tissues (detection limit 30 ng/ml).

Plasma levels for moxifloxacin 0.25 hours after dosing on day 6 were 0.171, 0.734, 2.61, and 8.13 µg/ml for 0.5, 2, 6.5, and 20 mg/kg doses. AUC for each dose was estimated from time 0 until the last blood sample with detectable moxifloxacin (7 hours for the lowest dose and 24 hours for the others). The day 6 AUCs for 0.5, 2, 6.5, and 20 mg/kg were 0.431, 2.48, 8.54, and 25.6 µg·hr/ml, respectively. The half life of moxifloxacin in the does was 2.2 hours at the lowest dose and 7-10 hours at the higher doses. Plasma levels for moxifloxacin 0.25 hours after dosing on day 19 were 0.176, 0.889, 2.85, and 10.0 µg/ml for 0.5, 2, 6.5, and 20 mg/kg doses. The day 19 AUCs for 0.5, 2, 6.5, and 20 mg/kg were 0.429, 3.17, 10.3, and 45.5 µg·hr/ml, respectively.

The maternal plasma concentrations measured 0.25 hours after dosing on day 20 were 0.173, 0.905, 2.92, and 9.17 µg/ml for 0.5, 2, 6.5, and 20 mg/kg doses. The fetal concentrations of moxifloxacin were 0.174, 0.940, 2.58 and 6.90 µg/g for the 0.5, 2, 6.5, and 20 mg/kg dose groups. These were 75-100% of the maternal plasma levels.

BAY 12-8039 Oral (Gavage) Embryo-Fetal Developmental Study in the Cynomolgus Monkey (Segment II) (Bayer Report No. R 7023; CHE Study No. 262-086; Study No. T 0054290)

[redacted]

Report dated: 12/24/97, [redacted]

Vol. 41, pp. 1-304

Animals: Pregnant (confirmed by ultrasound during days 18-20 of gestation) cynomolgus monkeys (purpose bred, 2.8-5.8 kg, at least 3 years old), 16 per dose group

Diet: [redacted] pelleted diet (50 g, twice daily), one slice of bread (once weekly), and fresh fruit (about twice weekly). Tap water was available *ad libitum*.

Drug Dose and Route of Administration: Moxifloxacin (Batch No. 960 704A) was suspended in 0.5% [redacted] and administered via intragastric intubation (10 ml/kg) daily from days 20-50 of gestation at doses of 0 (vehicle control) 10, 30, and 100 mg/kg.

Length and Conduct of Study: Fetuses were delivered via cesarean section on day 100 ± 1 of gestation. Blood samples were drawn from 5 monkeys per group on days 20 and 50 of gestation (days 1 and 31 of dosing) prior to dosing and 2, 4, 7, and 24 hours after dosing for toxicokinetic analysis (published in a separate report). Additionally, a final dose of moxifloxacin was given to the monkeys 4 hours prior to cesarean section. Blood samples were drawn from the maternal animals at the time of the cesarean section and from the umbilical vein of 5 fetuses per group after their delivery, prior to their sacrifice.

Monkeys were observed twice daily (once on weekends and holidays) for clinical signs. Food intake was estimated twice daily from day 20-100 of gestation and classified as normal or reduced. Vaginal smears were examined daily from day 20 until the day prior to cesarean section. Body weights were measured weekly from day 20 throughout gestation and on the day of cesarean section. Ultrasound was performed to monitor pregnancy every other week starting on day 30 of gestation. Additional ultrasonography was performed if animals showed signs of abortion.

Full necropsy was performed on each fetus and the viscera examined using stereomicroscopy. After most of the viscera were removed, the carcass containing skeletal remains was processed and stained for examination.

Results: One animal in the control group had a false positive pregnancy test and was replaced. None of the pregnant monkeys died during the experiment. None of the animals in the control or 10 mg/kg groups aborted. In the 30 mg/kg group, 3/16 monkeys aborted. Two of these animals aborted on day 44 and day 77, and the third had no live fetus detected on day 30, but the actual day of loss could not be determined. In the 100 mg/kg group, 4/16 aborted. One of these monkeys aborted on day 53 and the second between days 33-37. The actual day of loss could not be determined in the other 2 monkeys, but one had no live fetus detected in its uterus on days 44 and 47 and the other had no fetal heartbeat detected on day 44 with no live fetus detected on day 47 or 51. All of the abortions and fetal deaths occurred during the dosing period. In one control animal, a dead fetus (age appeared to be 98-99 days of gestation) was removed at the time of cesarean section. The rest of the fetuses were alive when cesarean section was performed.

Emesis was occasionally observed during the moxifloxacin administration period in 2 monkeys of the 30 mg/kg group and 11 monkeys of the 100 mg/kg group. Soft feces and/or diarrhea were observed in several animals from both of these dose groups during the drug treatment period.

Reduced food consumption was observed occasionally in all groups and did not appear to be related to moxifloxacin treatment. Mean body weight gain was similar between all groups.

The mean distance from coccyx to cranium was significantly shorter in fetuses from the 100 mg/kg group than control (11.7 ± 1.4 vs. 10.9 ± 0.7 cm). Mean fetal body weight was also less at 100 mg/kg than control (101.6 ± 21.6 vs. 91.5 ± 18.3 g), though the difference was not statistically significant. Despite the lack of statistical significance and the fact that the individual fetal body weights were within the historical control range, the investigators believed that the reduced fetal body weight in the 100 mg/kg groups was likely biologically significant because

almost all fetal measurement parameters in this treatment group were less than controls. Additionally, most of the individual fetuses in the 100 mg/kg group weighed less than most individual control fetuses- the difference between the 2 groups was consistent and not due to only one or two fetuses. Mean fetal organ weights (thymus, heart, lungs, liver, spleen, adrenal, kidney, brain, eye) at 100 mg/kg were also less than controls, though again, the differences were not statisticallly significant.

No moxifloxacin-related external, visceral, or skeletal malformations were observed in the fetuses. Several fetuses in each group (including control) had common external findings such as incomplete patency of prepuce, bent tail, and small ball of tissue at end of tail. One live control fetus had severe external, visceral, and skeletal malformations (curly tail, microophthalmia, abnormal positioned fingers, shortened toe, abnormally formed thoracic skeleton, fore- and hind limbs, small ovaries, absent ureters and urinary bladder, abnormal consistency of kidneys, adrenal glands, and spleen). All fetuses in each group had minor variations in skeletal ossification.

Doses of 30 and 100 mg/kg moxifloxacin given to pregnant cynomolgus monkeys were associated with dose-dependant increases in abortion frequency and maternal toxicity (vomiting, diarrhea). Fetal malformations were not observed at these doses, but fetuses from the 100 mg/kg group tended to be smaller than controls. The 10 mg/kg dose of moxifloxacin was not associated with maternal or fetal toxicity or teratogenicity.

BAY 12-8039: Plasma Concentrations in Pregnant Cynomolgus Monkeys in an Embryo Toxicity Study (Report No. PH 26931; Study No. T 6055259)

C. Kohlsdorfer, O. Mölders (Bayer, Wuppertal, Germany)

Report dated: 12/10/97

Vol. 49, pp. 75-99

Summary: This is the toxicokinetics portion of the dose setting study for developmental toxicity testing in cynomolgus monkeys. Five pregnant animals received 100 mg/kg of moxifloxacin daily from gestation days 20-50. Blood samples were drawn on the first and last days of the dosing period prior to administration of drug, then 2, 4, 7, and 24 hours after. On the day of cesarean section (day 100), the pregnant monkeys were given a final 100 mg/kg dose of moxifloxacin 4 hours before the procedure was performed. At the time of cesarean section, blood samples were drawn from the maternal monkeys and from the fetuses after delivery. A validated [redacted] was used to quantitate moxifloxacin in plasma (detection limit 5 ng/ml).

On day 20, the mean Cmax in the monkeys was 7.88 µg/ml and on day 50, it was 11.4 µg/ml. Tmax occurred between 2 and 7 hours after dosing. On day 20, the mean $AUC_{0\text{-}24\ hr}$ was 92.1 µg·hr/ml and on day 50 it was 154 µg·hr/ml. On day 50, moxifloxacin was detected in the plasma of all monkeys prior to dosing (mean of 2.95 µg/ml). These data demonstrate that accumulation of moxifloxacin occurred in these monkeys at oral doses of 100 mg/kg over 30 days. Half life on day 20 was abut 4-8 hours and on day 50 was 7-18 hours (drug had 7-9 hour half life in most monkeys on day 50, however).

The mean maternal plasma level 4 hours after 100 mg/kg was administered on the day of cesarean section was 5.74 µg/ml. The individual fetal plasma levels were 50.5-87.0% of the maternal levels.

BAY 12-8039: Plasma Concentrations in Cynomolgus Monkeys after Oral Administration in an Embryo-fetal Developmental Study- with Amendment (Report No. PH 27332; Study No. T 0054290)

C. Kohlsdorfer, B. Holzum (Bayer, Wuppertal, Germany)

Report dated: 3/10/98

Vol. 49, pp. 100-147

Summary: This is the toxicokinetics portion of the pivotal developmental toxicity study in cynomolgus monkeys (above). The amendment was to correct a typographical error.

Blood samples were drawn from 5 pregnant cynolmolgus monkeys per dose group (0, 10, 30, and 100 mg/kg) that received moxifloxacin from days 20-50 of gestation. Blood samples were drawn on the first and last days of the dosing period prior to administration of drug, then 2, 4, 7, and 24 hours after. On the day of cesarean section (day 100), the pregnant monkeys were given a final 100 mg/kg dose of moxifloxacin 4 hours before the procedure was performed. At the time of cesarean section, blood samples were drawn from the maternal monkeys and from the fetuses after delivery. A validated [redacted] was used to quantitate moxifloxacin in plasma (detection limit 5 ng/ml).

On day 20, the mean Cmax values in the monkeys at 10, 30, and 100 mg/kg were 0.899, 3.56, and 7.81 µg/ml and on day 50, they were 0.977, 3.50, and 9.84 µg/ml. Tmax occurred between 2 and 4 hours after dosing. On day 20, the mean $AUC_{0\text{-}24\ hr}$ values at 10, 30, and 100 mg/kg were 7.70, 30.8, and 86.6 µg·hr/ml and on day 50 they were 7.3, 33.4, and 120 µg·hr/ml. Based upon the AUC data, accumulation of moxifloxacin appeared to occur in these monkeys only at the high oral dose of 100 mg/kg over 30 days.

The mean maternal plasma levels 4 hours after 10, 30, or 100 mg/kg was administered on the day of cesarean section were 1.04, 3.21, and 4.12 µg/ml. The mean fetal plasma levels were 48.7-60.0% of the maternal levels.

Genotoxicity Studies:

BAY 12-8039: Mutagenicity Study for the Detection of Induced Forward Mutation in the CHO/HGPRT Assay *in vitro* (Bayer Report No. PH 26356; Study No. T 9053777)

S. Brendler-Schwaab (Bayer, Wuppertal, Germany)

Report dated: 2/5/97, [redacted]

Vol. 43, pp. 1-38

Method: Chinese hamster ovary cells (CHO-K_1-BH_4, cultured in hypoxanthine-free [redacted] medium supplemented with l-glutamine, penicillin/streptomycin, and fetal calf serum) were incubated with test substances (± S-9, as applicable) for 5 hours at 37°C. After the treatment period, the medium containing test substances was removed, fresh medium was added, and cultures were incubated for 7 days more. For selection of mutants at the HGPRT locus, cells were subcultured in duplicate in medium containing 6-thioguanine. After 6-7 days in the selection medium, cell colonies were fixed and stained for counting. The assay was conducted in both the presence and absence S-9 on at least 2 separate occasions to confirm results. The concentrations of moxifloxacin (Batch No. 519481) used for the mutagenicity studies were chosen based on the results of a preliminary cytotoxicity test under the same culture conditions. The highest dose levels were those demonstrating less than 30% survival of the CHO cells in the cytotoxicity assay. The concentrations of moxifloxacin used in the absence of metabolic activation were 62.5, 125, 250, 500, 750, and 1000 µg/ml and the concentrations used with S-9 were 125, 250, 500,750, 1000 and 1250 µg/ml. The S-9 assay was repeated using the same concentrations as the nonactivated assay due to excessive cytotoxicity and poor performance of the positive control. Ethyl methanesulfonate (EMS, 900 µg/ml) was the positive control in the absence of metabolic activation and dimethylbenzanthracene (DMBA, 20 µg/ml) was the positive control in the presence of S-9. The vehicle for moxifloxacin was deionized water and the vehicles for the positive controls were deionized water (EMS) and DMSO (DMBA). The negative control was untreated and a vehicle control (deionized water) was also used. The S-9 mix was derived from livers of male Wistar rats which had been treated with [redacted]

Results: A total of 4 acceptable independent assays were conducted with the CHO cells at moxifloxacin concentrations from 62.5-1000 µg/ml, 2 in the absence of metabolic activation and the others in the presence of S-9. Under these testing conditions, moxifloxacin did not appear to induce mutations at the HGPRT locus of CHO cells in the presence or absence of S-9.

BAY 12-8039: Mutagenicity Study for the Detection of Induced Forward Mutations in the V79-HGPRT Assay *in vitro* (Bayer Report No. PH 26367; Study No. T 5053700)

S. Brendler-Schwaab (Bayer, Wuppertal, Germany)

Report dated: 3/17/97; [redacted]

Vol. 43, pp. 39-79

Method: Chinese hamster lung cells (V79, cultured in hypoxanthine-free [redacted] minimal essential medium supplemented with nonessential amino acids, l-glutamine, vitamins, $NaHCO_3$, penicillin/streptomycin, and fetal calf serum) were incubated with test substances (± S-9, as applicable) for 5 hours at 37°C. After the treatment period, the medium containing test substances was removed, fresh medium was added, and cultures were incubated for 7 days more. For selection of mutants at the HGPRT locus, cells were subcultured in duplicate in medium containing 6-thioguanine. After 6-7 days in the selection medium, cell colonies were fixed and stained for counting. The assay was conducted in both the presence and absence of S-9 on 4 separate occasions to try to confirm results. The concentrations of moxifloxacin (Batch No.

519481) used for the mutagenicity studies were chosen based on the results of a preliminary cytotoxicity test under the same culture conditions. The highest dose levels were those demonstrating less than 30% survival of the CHO cells in the cytotoxicity assay. The concentrations of moxifloxacin used in the absence of metabolic activation were 50-1250 µg/ml and the concentrations used with S-9 were 125-1250 µg/ml, with 6 concentrations used per assay. Ethyl methanesulfonate (EMS, 900 µg/ml) was the positive control in the absence of metabolic activation and dimethylbenzanthracene (DMBA, 20 µg/ml) was the positive control in the presence of S-9. The vehicle for moxifloxacin was deionized water and the vehicles for the positive controls were deionized water (EMS) and DMSO (DMBA). The negative control was untreated and a vehicle control (deionized water) was also used. The S-9 mix was derived from livers of male Wistar rats which had been treated with [redacted]

Results: A total of 8 independent assays were conducted with moxifloxacin, 4 in the absence of metabolic activation and the others in the presence of S-9. Positive controls performed as expected in the experiments, but results with moxifloxacin were not consistent.

In the first nonactivation assay with moxifloxacin, very small increases in mutation frequency were observed in one replicate culture at almost all concentrations; however, there was no clear dose/response. A second trial appeared to be negative- only one replicate at 500 µg had a small increase in mutation frequency compared to control and no increase was observed at higher concentrations (up to 1000 µg/ml) despite adequate survival and cloning efficiency. A third experiment showed moderately increased mutation frequencies at all concentrations tested that had adequate survival and cloning efficiency (125-750 µg/ml), but there was no dose/response effect. The final experiment in the absence of metabolic activation showed modest increases in mutation frequency in one replicate at 250-750 µg/ml, but without a dose/response. Higher concentrations were not cloned in selection medium due to cytotoxicity.

In the presence of S-9, the first assay showed small increases in mutation frequency at 125, 500, and 750 µg/ml, but in only one replicate. At 1000 µg/ml, only one replicate was cultured in selection medium due to excessive cytotoxicity in the other replicate, but no increase was observed in the cultured replicate. In the second S-9 assay, both replicate cultures at 400 and 1000 µg/ml showed modest increases in mutation frequency, but no increase was seen at 600 or 800 µg/ml (only one replicate available for each due to contamination). In the third assay, only one replicate culture at 800 µg/ml showed an increase in mutation frequency- no other increases were observed. No data at higher concentrations were available in this assay due to excessive cytotoxicity. In the final assay conducted in the presence of S-9, one replicate at 400 µg/ml and both replicates at 800 µg/ml had modest increases in mutation frequency.

The investigators considered the overall response of moxifloxacin to be equivocal in this test system. There were increased mutation frequencies in both the absence and presence of S-9, but these tended to be very modest and a dose/response effect was not observed despite the repetition of the assay. Even when all of the data are considered together, a dose-related increase in mutation frequency is not apparent. The reviewer agrees that the results of this V-79/HGPRT assay were equivocal. Most of the increased in mutation frequency that were observed in the moxifloxacin-treated cultures were barely greater than the high end of this laboratory's historical negative control range for these cells. The individual positive cultures cannot be discounted completely, but no clear dose/response was observed and the results were not reproducible, although the investigators attempted to do so.

BAY 12-8039: Dominant Lethal Test on the Male Mouse (Bayer Report No. PH 25873; Study No. T 2060925)

B. Herbold (Bayer, Wuppertal, Germany)

Report dated: 10/22/96, [redacted]

Vol. 43, pp. 257-295

Animals: Bor:NMRI mice, 34-49 g, 9-13 weeks of age at the initiation of treatment and/or cohabitation, 50 males per treatment group for the definitive study

Diet: [redacted] Diet and water were provided *ad libitum*.

Drug Dose and Route of Administration: Moxifloxacin (Batch No. 519481) was suspended in 0.5% Cremophor emulsion and administered orally (10 ml/kg) once only to male mice at doses of 800 or 1200 mg/kg. These doses were chosen based upon the results of a pilot study in male mice where mortality was seen at doses >1600 mg/kg. A control group received vehicle. No concurrent positive control was used, but the report stated that this strain of mouse is sensitive to the dominant lethal effects of mutagenic chemicals such as cyclophosphamide and methyl methane sulfonate.

The males were mated with an untreated females for a period of 4 days for 48 consecutive days (total of 12 mating periods). The first mating period began on the day of drug treatment.

Length of Study: Female mice were sacrificed approximately 14 days after their assigned mating period. The numbers of corpora lutea, implantation sites, live, dead, and resorbed fetuses were counted and pre- and postimplantation loss were calculated.

Results: Clinical signs of toxicity were observed in the moxifloxacin-treated male mice for up to 4 days after administration. These included apathy, rough fur, staggering gain, sternal recumbency, spasm, difficult breathing and slit eyes. Three of the 1200 mg/kg males died within several days of dosing and their deaths were attributed to moxifloxacin toxicity by the investigators.

During the first mating period, fertilization rate was slightly lower in the 1200 mg/kg males than controls (74% vs. 64%). This was attributed to the toxic effects of moxifloxacin interfering with mating ability of the males. In subsequent mating periods, fertilization rate was not reduced in either moxifloxacin treatment group. At no time in the entire breeding period was either moxifloxacin dose associated with significantly greater numbers of dead or resorbed fetuses than controls. Additionally, the drug did not increase pre- or postimplantation loss at either dose levels tested.

Moxifloxacin demonstrated no evidence of a dominant lethal effect when a single oral dose of up to 1200 mg/kg was given to male mice.

Studies of Photostability and Lack of Photogenotoxicity of Moxifloxacin (Bayer Report No. R 7142)

[redacted]

Report dated: 5/28/98[redacted]

Vol. 29, pp. 121-137

Summary: A series of experiments was conducted with ciprofloxacin, lomefloxacin, BAY y3118 and moxifloxacin.

A 50 µM solution of each fluoroquinolone was irradiated with UVA[redacted] or UVB [redacted] for various times (to give UVA doses of 0-40 J or UVB doses of 0-4 J), then the concentration (absorbence) was re-checked with a spectrophotometer. UVA-induced photodecomposition of moxifloxacin was slowest among the fluoroquinolones tested and it was linear. The rate of UVA-induced photodecomposition of ciprofloxacin was slightly faster than moxifloxacin, and it was also linear. The UVA-induced photodecomposition rates of BAY y3118 and lomefloxacin were much more rapid than either moxifloxacin or ciprofloxacin, and they appeared biphasic (fast initial decomposition, followed by a slower decrease). Lomefloxacin appeared slightly less stable than BAY y3118. The results for UVB were qualitatively similar.

Oxidative damage induced by the fluoroquinolones in conjunction with UVA or UVB exposure was investigated using 0.1 mg/ml solutions of calf thymus DNA in 50 mM sodium phosphate buffer (pH 7.0). Test compounds were added 20 minutes prior to irradiation. Solutions were stirred during irradiation. Following the UVA or UVB exposure, DNA was precipitated then processed so that 8-oxo-2'-deoxyguanosine (8-oxo-dG) could be detected via [redacted] With UVA (20 J of radiation and various concentrations of each fluoroquinolone from 0-400 µM), BAY y3118 produced by far the most 8-oxo-dG per concentration level, followed by lomefloxacin. Ciprofloxacin produced some 8-oxo-dG at 50-200 µM, but the amount fell as concentrations were raised higher than 100 µM. Moxifloxacin produced little 8-oxo-dG over the entire concentration range. When 50 µM solutions of each quinolone were irradiated with UVB at doses of 0-4 J, lomefloxacin produced the most 8-oxo-dG, followed by BAY y3118. The production of 8-oxo-dG by these compounds leveled off after about 0.75 J of exposure. Ciprofloxacin did not produce as much 8-oxo-dG as either lomefloxacin or BAY y3118, but the production of the molecule by ciprofloxacin in the presence of UVB increased slowly, but steadily, at exposures up to about 2.25 J. Moxifloxacin did not produce detectable amounts of 8-oxo-dG in the presence of UVB up to about 4 J.

Fluoroquinolones (50 µM) were incubated with [redacted] to see whether strand breaks occurred in the presence of drug with UVA or UVB radiation. If the plasmid DNA is nicked, it converts from a supercoiled state to a relaxed, closed circular state. An agarose gel containing ethidium bromide was used to separate the 2 forms of DNA. In the presence of 0-100 mJ of UVA, moxifloxacin and ciprofloxacin produced fewer strand breaks than lomefloxacin and BAY y3118. Moxifloxacin plus UVB exposure (0-100 mJ) did not induce strand breaks in this model. At UVB doses of about 12.5 mJ and below, ciprofloxacin caused

fewer strand breaks than BAY y3118, but ciprofloxacin caused more strand breaks at UVB doses ≥25 mJ. Lomefloxacin was by far the most potent inducer of strand breakage with UVB, with most DNA converted from the supercoiled to the relaxed form at UVB doses below 10 mJ.

Moxifloxacin is more photostable and induced less oxidative DNA damage or DNA strand breakage in the presence of UVA or UVB than ciprofloxacin, BAY y3118, or lomefloxacin.

Photomutagenicity Studies of Moxifloxacin in Comparison to Other Fluoroquinolones (Bayer Report No. R 7143)

[redacted]

Report dated: 5/28/98, [redacted]

Vol. 29, pp. 138-154

Summary: The capacity of fluoroquinolones to induce DNA mutation at the HGPRT locus of V79 cells was investigated. V79-4 Chinese hamster lung cells were incubated with moxifloxacin (50 or 100 μM), BAY y3118 (56.5 μM), or lomefloxacin (400 μM) in serum [redacted] medium for one hour, then exposed to UVA [redacted] The UVA doses for lomefloxacin were 3000, 6000, and 9000 J/m^2; for moxifloxacin were 3000 and 9000 J/m^2; and for BAY y3118 was 1000 J/m^2. Lomefloxacin containing-medium was changed after 2 minutes in UVA due to its instability during UVA irradiation. After irradiation, cells were placed in an incubator for 5 hours longer before removal of medium containing drug and addition of fresh DEM containing 10% fetal calf serum. A positive control in the absence of UVA was 1 mM N-nitroso-N-methylurea (MNU). After treatment, cells were cultured for a week to allow expression. V79 cells were then incubated with 6-thioguanine to select mutants. After 9-10 days, plates were stained and colonies counted.

Under the conditions of this study, BAY y3118 was mutagenic at the HGPRT locus of V79-4 cells in the presence of UVA. Lomefloxacin was photomutagenic as long as fresh medium containing the drug was added to the cells 2 minutes after irradiation began; it is not very stable when exposed to UVA. Moxifloxacin did not appear to be photomutagenic under the conditions of this study, and it was stable under UVA.

Pharmacokinetic (ADME) Studies:

BAY 12-8039: Plasma Concentrations in Wistar Rats After Oral Administration in a 13-Week Toxicity Study (Bayer Report No. PH 25711; Study No. T 2060277)

C. Kohlsdorfer, K.H. Leser (Bayer, Wuppertal, Germany)

Report dated: 12/6/96

Vol. 17, pp. 197-236

Summary: This is the toxicokinetic portion of a study that was conducted in male and female (5/sex per group for TK) Wistar rats where moxifloxacin was given once daily via oral gavage for 13 weeks at doses of 0, 20, 100, 500 and 750 mg/kg. On days 1, 27, and 92 blood samples were drawn from the 20 mg/kg group 0.167 and 6 hours after dosing and from the other groups 1.5 and 24 hours after dosing so that plasma concentrations of moxifloxacin could be measured [redacted] Plasma samples were also collected on day 92 (after the last dose) for the rats in the recovery group. Females had lower plasma concentrations than males.

Average Moxifloxacin Plasma Concentrations in Rats After Oral Dosing

Dose	Time (hr)	Day 1		Day 27		Day 92	
		Male	Female	Male	Female	Male	Female
20 mg/kg	0.167	0.567	0.352	0.907	0.372	1.22	0.480
	6	0.128	0.025	0.378	0.030	0.279	0.031
100 mg/kg	1.5	3.22	0.498	2.65	0.370	2.29	0.756
	24	0.038	BLQ	0.097	BLQ	0.066	BLQ
500 mg/kg	1.5	8.14	3.08	5.32	1.70	7.45	2.95
	24	0.640	BLQ	1.57	0.029	1.20	0.077
750 mg/kg	1.5	9.94	4.83	7.85	2.42	14.5	5.53
	24	1.51	0.032	2.08	0.070	3.78	0.240
750 mg/kg (rec)	1.5	---	---	---	---	15.8	4.43
	24	---	---	---	---	4.57	0.241

BLQ, below limit of quantification

BAY 12-8039: Plasma Concentrations in Rhesus Monkeys After Oral Administration in a Subchronic Toxicity Study (Bayer Report No. PH 25706; Study No. T 5060045)

C. Kohlsdorfer, J. Ruf (Bayer, Wuppertal, Germany)

Report dated: 12/4/96

Vol. 25, pp. 447-493

Summary: This is the toxicokinetic portion of a study that was conducted in male and female (4/sex per group) Rhesus monkeys where moxifloxacin was given once daily via oral administration for 13 weeks at doses of 0, 15, 45, and 135 mg/kg. On days 1, 22, and 86, blood samples were drawn prior to dosing and 2, 4, 7, and 24 hours after administration so that plasma concentrations of moxifloxacin could be measured [redacted] In general, female monkeys had lower plasma concentrations than males. At the high dose, moxifloxacin had a moderate potential for accumulation in Rhesus monkeys.

Mean Toxicokinetic Parameters in Rhesus Monkeys After Oral Moxifloxacin Administration

	15 mg/kg		45 mg/kg		135 mg/kg	
	Male	Female	Male	Female	Male	Female
Day 1						
AUC_{0-24} (µg·hr/ml)	13.5	10.1	41.9	32.8	118	121
Cmax (µg/ml)	2.17	1.95	5.41	4.71	10.0	11.4
Tmax (hr)	2.00	2.38	2.38	2.83	4.60	4.00
Day 22						
AUC (µg·hr/ml)	14.6	9.92	41.2	45.7	166	134
Cmax (µg/ml)	2.24	1.56	5.01	5.53	13.5	11.0
Tmax (hr)	2.00	2.00	4.00	3.87	4.60	4.00
Day 26						
AUC (µg·hr/ml)	18.8	10.8	57.8	35.8	256	178
Cmax (µg/ml)	2.35	1.53	6.36	4.77	16.9	12.1
Tmax (hr)	3.36	2.38	4.00	4.00	7.00	7.00

BAY 12-8039: Plasma Concentrations in Rhesus Monkeys After Intravenous Infusion in a Subacute Toxicity Study (Bayer Report No. PH 25730; Study No. T 1060717)

C. Kohlsdorfer, J. Ruf (Bayer, Wuppertal, Germany)

Report dated: 12/11/96

Vol. 29, pp. 1-31

Summary: This is the toxicokinetic portion of a study that was conducted in male and female (3/sex per group) Rhesus monkeys where moxifloxacin was given once daily via 50 minute IV infusions for 4 weeks at doses of 0, 200, and 400 mg per animal (approximately 0, 36.4 and 76.0 mg/kg). On days 1 and 26, blood samples were drawn prior to dosing and 0.5, 1, 3, 7, and 24 hours after the start of infusion so that plasma concentrations of moxifloxacin could be measured [redacted] Both males and females had similar plasma concentrations, so the data for both genders were combined. The data demonstrate little potential for moxifloxacin accumulation in Rhesus monkeys at these doses.

Mean Toxicokinetic Parameters in Rhesus Monkeys After IV Moxifloxacin Doses of 200 or 400 mg/Animal

	200 mg	400 mg
Day 1		
AUC_{0-24} (µg·hr/ml)	65.1	137
Cmax (µg/ml)	9.53	21.4
Tmax (hr)	1	1

Day 26		
AUC (μg·hr/ml)	65.0	148
Cmax (μg/ml)	9.90	18.4
Tmax (hr)	1	1

[^{14}C]BAY 12-8039: Secretion of Radioactivity into Milk of Lactating Rats after a Single Oral Administration (Bayer Report No. PH 27004)

H.M. Siefert, H.P. Daehler, C. Kohlsdorfer (Bayer, Wuppertal, Germany)

Report dated: 1/8/98

Vol. 47, pp. 211-245

Summary: A 5 mg/kg dose of [^{14}C]BAY 12-8039 (Batch Nos. SXD 667-01-1H and SXD 667-01-1O) mixed with unlabeled moxifloxacin (Batch Nos. R-54-3 and R-55-1) dissolved in physiologic saline was administered orally to lactating Wistar rats (about 8 days post partum). Milk and plasma samples were collected from 5-6 rats per time point 1, 2, 4, 6, 8, and 24 hours after dosing and the amount of radiolabel was determined via liquid scintillation counting. [redacted] was also used on some samples to determine the amounts of unchanged moxifloxacin in the samples. Radioactivity (above background level) could no longer be detected in the milk 24 hours after administration. The amounts of radioactivity or unchanged moxifloxacin in plasma were much greater than the amounts in milk at all of the time points studied. For the time interval of 1-8 hours, the half life of radioactivity in milk and plasma was similar (2.0 vs. 2.2 hours). The maximum level of radioactivity present in milk or plasma occurred 1-2 hours after administration. The mean $AUC_{0\text{-}8\ hr}$ values for milk and plasma were 0.164 and 0.354 μg eq·hr/ml. [redacted] indicated that about 20-30% of the amount of radioactivity in the milk at the 1 and 2 hour time points was unchanged moxifloxacin. Overall, the sponsor estimated that only about 0.03% of the total radioactive dose of moxifloxacin was excreted in the milk over 24 hours (assuming continuous daily milk production of about 20% of maternal body weight).

Radiosynthesis of [^{14}C]BAY 12-8039 (Bayer Report No. PH 24396)

and

Second Radiosynthesis of [^{14}C]BAY 12-8039 (Bayer Report No. PH 25747)

These reports describe a method for synthesizing radiolabeled moxifloxacin and a refinement to the original method that gave the chemists a better yield of the desired product, 1-cyclopropyl-7-[(S,S)-2,8-diazabicyclo-[4.3.0]non-8-yl]-6-fluoro-8-methoxy-4-oxo-1,4-dihydro-[3-^{14}C]quinoline-3-carboxylic acid hydrochloride.

Absorption and Excretion of Substance Associated Radioactivity in Female Rats (Bayer Report No. PH 27336)

H.M. Siefert, H.P. Daehler (Bayer, Wuppertal, Germany)

Report dated: 3/25/98

Vol. 45, pp. 152-173

Summary: Radiolabeled moxifloxacin (Batch Nos. SXD 667-01-1J and SXD 667-01-1F) was given to groups of 5 female HSD/Cpb:WU rats (8 weeks old) at a single dose of 5 mg/kg. The drug was administered intravenously to intact rats and intraduodenally to bile-duct cannulated rats (10 ml/kg dose volume IV and 1 ml/kg intraduodenal). The rats were fasted for 16 hours prior to administration of drug and given food [redacted] 4 hours after dosing. The amount of radiolabel in the feces and urine collected for 48 hours after dosing was quantitated after IV dosing and the amount of radiolabel in excretory products and bile collected for 24 hours after dosing was quantitated.

Following intravenous administration to intact female rats, about 96% of the radiolabel was excreted in the feces and 5.34% was excreted in urine over 48 hours after dosing.

After intraduodenal administration to the bile duct cannulated rats, 80.5% of the dose was excreted in bile, 4.9% was excreted in urine, and 20.6% was excreted in the feces over 24 hours after dosing. Most of the biliary excretion occurred within the first 8 hours after dosing.

[^{14}C]BAY 12-8039: Investigation of the Enterohepatic Circulation in Male Rats (Bayer Report No. 27344)

A. Witt, A. Kern (Bayer, Wuppertal, Germany)

Report dated: 3/27/98

Vol. 45, pp. 231-255

Summary: Radiolabeled moxifloxacin (Batch No. SXD 0667-01-1E, in saline) at a dose of 5 mg/kg was administered intraduodenally (1 ml/kg) to 3 fasted bile duct cannulated male HsdCpb:WU rats (8 weeks old). The bile was collected from these rats for 24 hours. The amount of radiolabel in the bile was determined and then the bile was administered intraduodenally to a second group of 3 rats. Urine and feces from both groups of rats were collected for 24 hours after administration of radiolabeled drug or bile.

In the first group, 46.1% of the dose was excreted into bile, 46.6% was excreted into feces, and 6.3% was found in the urine. Most of the radiolabel present in the bile was identified as the N-sulfate metabolite of moxifloxacin, with a small amount of the glucuronide conjugate of moxifloxacin as well as the glucuronide conjugate of the N-sulfate. Less than 1% of the total dose was found in the bile as unchanged moxifloxacin. In the second group of rats, the dose was almost completely excreted into the feces, with less than 0.5% in the urine, less than 2% in the

bile, and little remaining in the body (less than 1.5%). Enterohepatic circulation of moxifloxacin was minimal in the male rat.

BAY 12-8039: Pharmacokinetics of the Unchanged Compound in Wistar Rats after Single Intra-Colonic Administration (Bayer Report No. PH 26635)

H.M. Siefert, C. Kohlsdorfer (Bayer, Wuppertal, Germany)

Report dated: 9/17/97

Vol. 46, pp. 1-37

Summary: Fasted male rats (HsdCpb:WU, 8 weeks old) received a single intracolonic 10 mg/kg dose (1 ml/kg) of moxifloxacin (Batch No. 521724E) in normal saline. Blood samples were drawn 5, 15, and 30 minutes and 1, 1.5, 2, 3, 4, 6, 8, and 24 hours after dosing so that the plasma concentration of moxifloxacin could be determined. Four animals were sampled at each time point. Measurable plasma levels could be detected as early as 5 minutes after drug was administered, with the Tmax around 15-90 minutes after dosing. The average pharmacokinetic parameters were: Cmax, 0.376 µg/ml; AUC, 1.92 µg·hr/ml; and half life about 6 hours. The investigators used some data from an intravenous study conducted in the same strain of rats to estimate the absolute bioavailability of moxifloxacin at 53% when administered into the colon. The data indicate that rats can absorb moxifloxacin from the lower portions of the GI tract.

[^{14}C]BAY 12-8039: Biotransformation in Wistar Rat (Bayer Report No. PH 27324)

A. Kern, M. Blombach, H.M. Siefert, R. Froede (Bayer, Wuppertal, Germany)

Report dated: 3/20/98

Vol. 46, pp. 131a-191

Summary: Radiolabeled moxifloxacin was administered as a single 5 mg/kg dose to rats via oral (males), intravenous (males and females, some males had bile duct cannulas), and intraduodenal (females with bile duct cannulas) routes. Samples of plasma (collected 1 and 8 hours after oral dosing and 10 minutes and 1 hour after IV dosing), urine (collected for 24 hours at 4 hour intervals), bile, and fecal extracts (feces collected for 24 hours after dosing from bile duct cannulated males only) were analyzed for moxifloxacin and metabolites.

After oral or intravenous dosing, the majority of the radiolabel in plasma (79.7-100%) was unchanged moxifloxacin, with up to 20.4% the acyl glucuronide metabolite (M2).

After oral and intravenous dosing to intact male rats, 11.7% and 14.4% of the administered radiolabel was found in the urine within 24 hours. In male (IV dosing) and female (intraduodenal dosing) rats with bile duct cannulas, 9.3% and 5.4% of the total dose was found in urine. Most of the radiolabel in the urine was unchanged moxifloxacin (65-81% of the radiolabel in al males and intact females, and 32% in bile duct cannulated females). About 12-15% of the

radiolabel in intact males was M2, with other metabolites making up less than 5%. In intact females, less than 2% of the administered dose was excreted as urinary metabolites.

In the bile duct cannulated rats, 76% of the dose was found in the bile of males after IV administration and 81% of the dose was found in the bile of females after intraduodenal dosing. In males, unmetabolized moxifloxacin in the bile was equal to about 0.88% of the dose and in females it was equal to 0.22%. Most of the radiolabel in the bile (80% in males and 94% in females) was the N-sulfate conjugate of moxifloxacin (M1). Other metabolites detected were M2 (about 7.3% of the dose in males and 2% in females), M3 (the acyl glucuronide of the N-sulfate, about 3% of the dose in both genders), and a small amount of M5 (an oxo metabolite of moxifloxacin) was found in males only.

Metabolites of moxifloxacin could not be detected in the feces of male bile duct cannulated rats. About 14% of the radioactive dose administered to these animals via the IV route was detected in the fecal extracts.

BAY 12-8039: Dose Dependence of Pharmacokinetics in Female Rhesus Monkeys After Single Intravenous Infusion (Bayer Report No. PH 26663)

H.M. Siefert, C. Kohlsdorfer (Bayer, Wuppertal, Germany)

Report dated: 9/24/97

Vol. 46, pp. 192-232

Summary: Moxifloxacin (unlabeled Batch No. 950710A, radiolabeled Batch Nos. 514271 and CMI25801-7C) was administered intravenously (15 minute infusion) at doses of 0.3, 3, and 15 mg/kg to 3 fasted female Rhesus monkeys on three separate occasions (at least 2 months apart). Blood samples were drawn 5 minutes to 31.25 hours after administration of the two lower doses and 5 minutes to 55.25 hours after administration of the high dose. Plasma concentrations rose dose-dependently, but AUC values rose slightly more than dose proportionately.

Mean Pharmacokinetic Parameters of Moxifloxacin in Female Rhesus Monkeys After IV Dosing

	0.3 mg/kg	3 mg/kg	15 mg/kg
AUC (μg·hr/ml)	0.284	3.98	25.2
Cmax (μg/ml)	0.102	1.13	8.15
$T_{1/2}$ (hr)	2.95	3.43	3.1
Vss (l/kg)	3.93	4.85	4.05
Clearance (l/hr·kg)	0.967	0.691	0.546

[^{14}C]BAY 12-8039: Distribution to Organs/Tissues of Male Wistar Rats After Single Oral Administration (Bayer Report No. PH 27223)

H.M. Siefert, G. Goeller (Bayer, Wuppertal, Germany)

Report dated: 2/20/98

Vol. 47, pp. 1-49

Summary: Radiolabeled moxifloxacin (Batch No. CMI 258-01-5A diluted with nonlabeled Batch No. 514271) was administered orally to fasted male rats (HsdCpb:WU, 8 weeks old) at a dose of 5 mg/kg. Animals were sacrificed and tissue samples obtained 0.5, 1, 4, 8, 24, 48, 72, and 168 hours after administration. There were 5 rats per time point.

Plasma protein binding was determined via ultrafiltration. The fraction unbound during the first 8 hours after dosing was 65-69%. It decreased to approximately 23% 24 hours after dosing, perhaps indicating greater binding of a metabolite.

Radiolabel had distributed to most tissues 0.5 hour after dosing. This time was the Tmax for many tissues. Tissues that had concentrations of radiolabel at Cmax greater than those found in plasma were (in descending order): kidney, liver, salivary gland, spleen, femur, adrenal, bone marrow, prostate, skeletal muscle, heart and its vessels, thyroid, skeletal muscle, lung, skin, and cartilage. Tissues that had Cmax comparable to plasma included adipose, xiphoid process, eye, and red blood cells. The testes, brain, and vitreous humor of the eye had levels lower than plasma at Cmax. By 7 days after dosing, radioactive residues remained in the skin at low levels.

[^{14}C]BAY 12-8039: Whole-Body Autoradiography in Female Rats After Intravenous Administration (Study No. I 3000922) (Bayer Report No. 26860)

W. Steinke, K. Heidenreich (Bayer, Wuppertal, Germany)

Report dated: 11/25/97

Vol. 47, pp. 98-126

Summary: Radiolabeled moxifloxacin (Batch No. SXD 0667-01-1A) was administered intravenously to fasted female rats (HsdCpb:WU, 8 weeks old) at a dose of 5 mg/kg. One rat per time point was sacrificed 5 minutes, 1 hour and 24 hours after administration of drug. Animals were frozen and embedded in carboxymethyl cellulose so that sagital sections 50 µm thick could be cut and exposed to X-ray film for 45-90 days.

At the 5 minute time point, many tissues had greater concentrations of radiolabel than blood, indicating rapid distribution. The highest amounts of radioactivity were seen in the bile ducts, contents of small intestine, urinary bladder, and outer medulla of kidneys. Relatively high concentrations were also observed in adrenals, incisors, pineal body, pituitary, kidney, pancreas, myocardium, bone marrow, liver, GI mucosa, and spleen. The female genital organs, skeletal muscle, thymus, and stomach contents also had higher concentrations than blood, but lower than the other organs mentioned previously. Tissues having similar concentrations to blood were

skin, cartilage, esophagus, trachea, nasal mucosa, epiphysis of bone, adipose, and connective tissue. Little radioactivity crossed the blood brain barrier, and none was observed in compact bone or the eye lens.

Tissue levels of radioactivity 1 hour after administration were lower than those observed 5 minutes after dosing, but distribution was similar. By 24 hours after dosing, radioactivity was no longer detected in many tissues, including the blood. Detectable levels of radioactivity were still observed in the peridental area of the incisors, the GI contents, intestinal mucosa, bile ducts, nasophayngeal mucosa, bone marrow, cartilage, skin, renal bladder, pineal body, thyroid, and liver.

[^{14}C]BAY 12-8039: Whole-Body Autoradiography in Pregnant Rats After Single Intravenous and Oral Administration (Study Nos. I 5000807 and I 8000981) (Bayer Report No. PH 27305)

W. Steinke, C. Crummenerl (Bayer, Wuppertal, Germany)

Report dated: 3/17/98

Vol. 47, pp. 127-172

Summary: Radiolabeled moxifloxacin (Batch Nos. CMI 258-01-7E and SXD 0667-01-1G, diluted for the IV study with nonradiolabeled Batch No. 514 271) was administered orally or intravenously to fasted pregnant rats (HsdCpb:WU, 12-14 weeks old, day 17-19 of gestation) at a dose of 5 mg/kg. One or two rats per time point were sacrificed 5 minutes or 1 hour after IV administration of drug or 1, 2, 4, 8, or 24 hours after oral dosing. Animals were frozen and embedded in carboxymethyl cellulose so that sagital sections 50 µm thick could be cut and exposed to X-ray film for 90-92 days.

As in nonpregnant rats, there was a rapid distribution of moxifloxacin to the tissues within 5 minutes after IV administration. There was penetration across the placental barrier, with some fetal tissues containing more radioactivity than maternal blood. Although radiolabel did not penetrate the blood brain barrier in the dams, that was not true for the fetuses, whose brains had levels of radioactivity similar to blood levels. Fetal tissues that had higher concentrations of radioactivity than maternal blood included liver, lungs, myocardium, cartilage, thymus, skeletal muscle, kidney, adrenal, skin, and eye. Tissue radiation levels in dams and fetuses fell within an hour after IV dosing, and the radioactivity remaining in the fetuses was distributed homogeneously.

The distribution pattern of radioactivity 1-8 hours after oral administration was qualitatively similar to that observed after IV dosing, but the amount of radiation was lower. Radioactivity was no longer detected in the fetuses 24 hours after oral administration.

[^{14}C]BAY 12-8039: Quantitative Organ Distribution and Placental Transfer of the Substance-Associated Radioactivity in Pregnant Wistar Rats After Single Oral Administration (Bayer Report No. PH 27189)

H.M. Siefert, H.P. Daehler (Bayer, Wuppertal, Germany)

Report dated: 2/11/98

Vol. 47, pp. 173-210

Summary: Radiolabeled moxifloxacin (Batch Nos. SXD 0667-01-1G and SXD 0667-01-1N) was administered orally fasted pregnant rats (HsdCpb:WU, 12-14 weeks old, day 17-18 of gestation) at a dose of 5 mg/kg. Four rats per time point were sacrificed and tissue samples were obtained 2, 4, 6, 8, 9, or 24 hours after dosing.

The maximum levels of radioactivity measured in maternal and fetal tissues occurred 2 hours after dosing. The highest maternal levels (in descending order) were found in liver, spleen, amnion, pancreas, kidneys, adrenal, and ovary. Other tissues that had greater levels of radioactivity than maternal plasma (but not as great as the tissues above) were thyroid, adipose, bone marrow, placenta, and thymus. Tissues that had similar levels of radioactivity compared to maternal plasma were skeletal muscle, lungs, heart, salivary gland, skin, femur, and eye. Maternal brain levels of radioactivity were very low. The amniotic fluid had a level of radioactivity similar to maternal plasma and the fetal plasma level was slightly lower than this. The amount of radioactivity in the fetal brain was similar to that in the amniotic fluid, indicating penetration of moxifloxacin through an immature blood/brain barrier. The fetal liver had a greater amount of radioactivity than fetal plasma. A small amount of radioactivity could still be detected in the fetuses 24 hours after to radiolabeled moxifloxacin dose was administered. The maternal tissues with the longest half lives for radiolabel were spleen, thyroid, and amnion.

BAY 12-8039: Concentrations of Unchanged Compound in Skin Suction Blister Fluid, Plasma, and Lung Tissue of Male Wistar Rats After Single Intravenous and Oral Administration (Bayer Report No. PH 27342)

H.M. Siefert, C. Kohlsdorfer, A. Witt (Bayer, Wuppertal, Germany)

Report dated: 3/27/98

Vol. 47, pp. 246-279

Summary: Radiolabeled moxifloxacin (Batch Nos. SXD 0667-01-01K, M, and Q diluted with nonlabeled Batch Nos. I 3001020/2 and 4 and I 8001061/1-2) was administered orally or intravenously to fasted male rats (HsdCpb:WU, 8 weeks old) at a dose of 5 mg/kg. Blood and lung tissue samples were obtained 0.5, 1.5, 3, 4.5, and 7 hours after administration. Blister fluid (generated by applying a vacuum to the flanks for one hour) was obtained 4.5 hours after dosing. There were 4 rats per time point.

The concentration of moxifloxacin in blister fluid 4.5 hours after dosing was 0.332 µg/ml after IV administration and 0.174 µg/ml after oral administration. Protein binding in blister fluid (67% unbound) was similar to that calculated for rat plasma in an earlier study (63% unbound).

Lung concentrations tended to be higher than plasma concentrations and received greater exposure (based upon AUC) although half life was similar in both plasma and lung.

Mean Moxifloxacin Pharmacokinetic Parameters in Rat Plasma and Lung Tissue Following an IV or Oral 5 mg/kg Dose

		IV	Oral
Plasma	AUC (µg·hr/ml)	2.5	1.49
	$T_{1/2}$ (hrs)	1.11	1.61
Lung	AUC (µg·hr/ml)	7.68	4.97
	$T_{1/2}$ (hrs)	0.88	1.43

[^{14}C]BAY 12-8039: Biotransformation in Rhesus Monkey (Bayer Report No. PH 27323)

A. Kern, M. Blombach, R. Froede, U. Petersen (Bayer, Wuppertal, Germany)

Report dated: 3/20/98

Vol. 47, pp. 280-348

Summary: Groups of 3 female rhesus monkeys received a 10 mg/kg oral dose or 3 mg/kg intravenous dose of radiolabeled moxifloxacin (CMI 258-01-7a, diluted with nonlabeled Batch 51 4271 for oral dosing). Plasma samples were taken 1, 4, 10 and 24 hours after oral dosing and 0.5, 1.25, and 4.25 hours after intravenous administration. Urine samples were collected at intervals up to 48 hours after dosing and fecal samples were collected up to 96 or 120 hours after drug was administered.

After oral administration, 18.3% of the radioactive dose was recovered in urine and 61.2% of the dose was recovered in the feces. After IV administration, 23.1% of the radiolabel was found in urine and 54.6% was found in feces.

In plasma, the majority of the radiolabel was unchanged drug (57.5-94%). The main metabolite in plasma was the acyl glucuronide (M2) and it accounted for up to 24.2% of the radiolabel. A number of minor metabolites (M1, M5, M6, M7, M9, and M4/8- unseparable in plasma) were also detected.

Unchanged moxifloxacin accounted for 26.3% of the radioactivity detected in urine. The major metabolites were M2 (15.8% of radioactivity), a moxifloxacin oxo-metabolite (M5, 3 and 10% of the urinary activity after oral and IV dosing), and the acyl glucuronide of the oxo-metabolite (M6, about 5% of the urinary radioactivity). Minor metabolites, M1, M4, M7, M8, and M9 (each making up 1% or less of the total dose) were also detected.

Unchanged moxifloxacin accounted for 8.7% or 6% of the radioactivity detected in feces after oral or IV administration, respectively. The major fecal metabolite was M1, the N-sulfate conjugate of moxifloxacin, accounting for 52-54% of the radioactivity in feces. M5 accounted

for about 18% of fecal radioactivity. M4 and M8 could not be separated in feces and together accounted for about 2-5% of the moxifloxacin dose. M7 was detected in feces only after oral administration and was a minor metabolite (0.15% of dose).

[^{14}C]BAY 12-8039: Biotransformation in Microsomal Liver Fractions and Hepatocytes of Rat, Monkey, and Man (Bayer Report No. Bayer Report No. PH 27341)

A. Kern (Bayer, Wuppertal, Germany)

Report dated: 3/27/98

Vol. 48, pp. 1-32

Summary: Several *in vitro* systems were used to explore the metabolism of radiolabeled moxifloxacin (Batch CMI 258-01-7a). Liver microsomes isolated from Rhesus monkeys and humans (capable of oxidative metabolism) did not metabolize moxifloxacin when reactions were allowed to sit for up to 3 hours. Isolated hepatocytes from male Wistar rats incubated with moxifloxacin for up to 4 hours (71% of drug was metabolized) produced primarily M1 and M2, with a minor amount of M3. Small amounts of M5 and M6 were seen only at the highest cell concentrations. Isolated hepatocytes from Rhesus monkeys incubated with drug for up to 4 hours produced primarily M1 and M2. The conjugate M6 and the phase I metabolites M4, M5, and M7 were also produced by the isolated monkey hepatocytes. Cultured primary hepatocytes from Wistar rats and humans produced M1 and M2 when incubated for up to 48 hours. A small amount of M3 was also detected in the rat culture. Cultured primary hepatocytes for Rhesus monkeys produced primarily M1, but M2, M5, and M6 were also detected. M4 and M7 were minor metabolites from the Rhesus hepatocytes.

M1: N-sulfate conjugate of moxifloxacin
M2: acyl glucuronide of moxifloxacin
M3: N-sulfate conjugate of acyl glucuronide
M4: hydroxy-metabolite of moxifloxacin
M5: oxo-metabolite of moxifloxacin
M6: acyl glucuronide of oxo-metabolite
M7: hydroxylated oxy-metabolite

BAY 12-8039: Chiral Inversion in Rats, Rhesus Monkey, and Man (Bayer Report No. PH 27325)

Kern, P. Mayer-Fligge (Bayer, Wuppertal, Germany)

Report dated: 3/20/98

Vol. 48, pp. 60-73

Summary: Moxifloxacin (BAY 12-8039) contains only an S,S-enantiomer. The molecule has a chiral center, so it is also possible to have an R,R-enantiomer (BAY 34-9117). Radiolabeled moxifloxacin was given to male rats (IV, 3 mg/kg), female Rhesus monkeys (IV, 3 mg/kg), and humans (oral, 600 mg). Urine was collected for 4 hours from the rats, 8 hours for the monkeys, and 24 hours for the humans. Only the S,S-enantiomer was detected in the urine of these species. No R,R-enantiomer was found. The investigators [redacted] to separate moxifloxacin and followed it with capillary electrophoresis with UV detection to separate the enantiomers (a small amount of the R,R enantiomer was added to some specimens to ensure that if that enantiomer was present, it would be separated and identified). Thus, chiral inversion of moxifloxacin does not appear to occur *in vivo* in rats, humans, or monkeys.

BAY 12-8039: Plasma and Skin Concentrations in Guinea Pig After Oral Single Dose Administration in Two Mimicking Phototoxicity Studies (Bayer Report No. 27440)

C. Kohlsdorfer, H.W. Vohr (Bayer, Wuppertal, Germany)

Report dated: 4/29/98

Vol. 49, pp. 219-270

Summary: This is the toxicokinetics report for studies T 2061249 and T 8061263. Female guinea pigs (Hsd POC:DH, 3 per dose group per time point) received single oral doses or 7 daily doses of 0, 100, 300, or 500 mg/kg moxifloxacin (Batch No. 950710B). The amount of drug given to the animals is expressed in terms of the HCl salt, in terms of free moxifloxacin the doses were 0, 87.1, 261, and 436 mg/kg. Blood and skin samples were taken 0.5 hours after the final dose of drug was given (prior to irradiation) or 1.5 hours after the final dose of drug was given (after irradiation was completed).

Mean Moxifloxacin Concentrations (µg/ml or µg/ml) in Plasma and Skin of Guinea Pigs 0.5 or 1.5 Hours After Oral Administration

	87.1 mg/kg		261 mg/kg		436 mg/kg	
Single Dose	**0.5 hr**	**1.5 hr**	**0.5 hr**	**1.5 hr**	**0.5 hr**	**1.5 hr**
Plasma	2.86	1.43	4.66	3.14	8.10	2.21
Skin	1.83	1.81	3.28	4.15	7.44	2.73
Day 7						
Plasma	3.13	1.75	6.01*	6.06*	18.0*	10.1**
Skin	1.57	1.65	3.33*	3.11*	7.34*	7.98**

*data from one animal due to unscheduled death of other 2 group members

** data from two animals due to unscheduled death of the 3rd group member

BAY 12-8039: Plasma Concentrations After Single Oral Administration of 1000 mg/kg BAY 12-8039 and 1000 mg/kg BAY 11-6371 to Male NMRI Mice (Bayer Report No. 27446)

H.M. Siefert, C. Kohlsdorfer (Bayer, Wuppertal, Germany)

Report dated: 4/30/98

Vol. 49, pp. 271-301

Summary: The systemic exposure of moxifloxacin given as the HCl salt or tosylate salt (BAY 12-8039 and BAY 11-6371, respectively) was determined after oral administration to male NMRI mice. The doses of the salts were 1000 mg/kg, which converted to doses of free moxifloxacin of 918 mg/kg for the HCl salt BAY 12-8039 (Batch No. 521724E) and 763 mg/kg for the tosylate salt BAY 11-6371 (Batch No. 514236). These doses were chosen because they had been used for mouse micronucleus studies in this species. The drugs were dissolved in a small amount of [redacted] (40-42 µl) and added to 7.9-8.3 ml of distilled water to create a suspension. The dose volume was 5-6 ml/kg. Blood samples were drawn from 3 mice per dose group 5, 10, 15, and 30 minutes and 1, 1.5, 2, 3, 4, 6, 8, and 24 hours after dosing for determination of moxifloxacin concentrations.

For BAY 12-8039, the Cmax was 26.4 µg/ml, Tmax was about 15 minutes, half life was 12.6 hours, and AUC was 80.5 µg·hr/ml. For BAY 11-6371, the Cmax was 12.6 µg/ml, Tmax was about 15 minutes, half life was 17.8 hours, and AUC was 137 µg·hr/ml. The hydrochloride salt of moxifloxacin was absorbed and excreted more rapidly than the tosylate form.

BAY 12-8039: Plasma Concentrations in Wistar Rats After Oral Administration in a 16 Day Mimicking Study (Bayer Report No. PH 27470; Study No. T 2061960)

C. Kohlsdorfer, K.H. Leser (Bayer, Wuppertal, Germany)

Report dated: 5/12/98

Vol. 51, pp. 1-97

Summary: BAY 12-8039 (Batch No. 522381D) was suspended in 0.5% [redacted] and administered via oral gavage to male and female Wistar rats (HsdCpb:WU, 3/sex/group) once daily for 15 days at doses of 0, 20, 100, 500, and 750 mg/kg. On days 1 and 15 of administration, blood samples were drawn 0.5, 1, 2, 4, 7, and 24 hours after dosing so that the plasma concentration of moxifloxacin could be measured.

Plasma concentrations were higher in male than female rats, consistent with other toxicokinetic studies in this species. Accumulation of moxifloxacin was not observed in rats at these doses over the 15 day study.

Mean Toxicokinetic Parameters in Rats After Moxifloxacin Administration

	20 mg/kg		100 mg/kg		500 mg/kg		750 mg/kg	
Day 1	**M**	**F**	**M**	**F**	**M**	**F**	**M**	**F**
Cmax (μg/ml)	1.64	0.302	4.53	1.28	11.3	5.61	12.5	6.35
Tmax (hr)	1	0.5	1	0.5	0.5	1	1	0.5
$T_{1/2}$ (hr)	3.2	2.8	3.6	2.5	11.3	2.5	24.8	2.8
AUC* (μg·hr/ml)	5.9	0.38	28.6	2.05	123	21.1	177	36.7
Day 15								
Cmax (μg/ml)	1.36	0.181	3.41	1.22	11.2	5.08	11.0	7.66
Tmax (hr)	0.5	0.5	1	0.5	0.5	0.5	7	0.5
$T_{1/2}$ (hr)	nc	nc	nc	nc	nc	nc	nc	nc
$AUC_{0\text{-}24\ hr}$ (μg·hr/ml)	6.09	0.31	24.0	1.98	125	14.3	164	31.9

nc, not calculated

*0-24 hours in males and females from 500 and 750 mg/kg dose groups and 0-7 hours in females from 20 and 100 mg/kg dose groups depending on last blood collection with detectable drug

RECOMMENDATIONS FOR LABEL: The reviewer's suggested deletions from the sponsor's proposal are struck out and suggested additions are in ***bold italic*** text.

Carcinogenesis, Mutagenesis, Impairment of Fertility:

Long term studies in animals to determine the carcinogenic potential of moxifloxacin have not been performed.

Moxifloxacin was not mutagenic in 4 ***bacterial*** strains (TA 98, TA 100, TA 1535, TA 1537) ***used in*** the Ames *Salmonella* reversion assay. As with other quinolones, the positive response observed with moxifloxacin in strain TA 102 ***using the same*** assay may be due to the inhibition of DNA gyrase. Moxifloxacin was not mutagenic in the CHO/HGPRT mammalian cell gene mutation assay. An equivocal result was obtained in the ***same*** assay ***when V79*** cells ***were used.*** Moxifloxacin was clastogenic in the *V79* chromosome aberration assay, ***but it did not induce*** ~~the~~ unscheduled DNA synthesis in ***cultured*** rat hepatocytes. There was no evidence of genotoxicity ***in vivo*** in a micronucleus test ***or a*** dominant lethal test ***in mice.***

Moxifloxacin had no effect on fertility in male and female rats at oral doses as high as 500 mg/kg/day ***approximately 12*** times the ***maximum*** recommended human dose ***based upon body surface area (mg/m^2).*** At 500 mg/kg there were slight effects on sperm morphology (head-tail separation) in male rats and on the estrous cycle in female rats.

Pregnancy: Teratogenic Effects. Pregnancy Category C:
Moxifloxacin was not teratogenic ***when administered to pregnant*** rats ***during organogenesis*** at oral doses as high as 500 mg/kg/day (***0.24 times the maximum recommended human dose based upon systemic exposure [AUC]***), ***but*** decreased fetal body weights and slightly delayed fetal skeletal development ***(indicative of fetotoxicity) were observed.***

Intravenous administration of 20 mg/kg/day (***approximately equal to the maximum recommended human dose based upon systemic exposure***

to ***pregnant*** rabbits ***during organogenesis*** resulted in decreased fetal body weights ~~***and***~~ delayed fetal skeletal ossification. ***When rib and vertebral malformations were combined, there was*** an increased ***fetal and litter*** incidence of ***these effects.***

Signs of maternal toxicity in rabbits at ***this dose*** included mortality, abortions, marked reduction of food consumption, decreased water intake, body weight loss and hypoactivity. There was no evidence of teratogenicity when ***pregnant*** Cynomolgus monkeys were ~~dosed~~ ***given oral doses*** as high as 100 mg/kg/day (***2.5 times the maximum recommended human dose based upon systemic exposure***

An increased incidence of smaller fetuses was observed at 100 mg/kg/day. In an oral pre ***and*** postnatal development study conducted in rats, effects observed at 500 mg/kg/day included ***slight increases in*** duration of pregnancy ***and*** prenatal loss, reduced ***pup*** birth weight and decreased ***neonatal*** survival. Treatment-related maternal mortality occurred ***during gestation*** at 500 mg/kg/day ***in this study***. Since there are no adequate or well-controlled studies in pregnant women, moxifloxacin should be used during pregnancy only if the potential benefit justifies ***the*** potential risk to the ***fetus***

Nursing Mothers: ***Moxifloxacin is excreted in the breast milk of rats*** ***Moxifloxacin*** ~~**is**~~ ***may also be*** excreted in human milk. Because of the potential for serious adverse reactions in infants nursing from mothers taking moxifloxacin, a decision should be made whether to discontinue nursing or to discontinue the drug, taking into account the importance of the drug to the mother.

OVERDOSAGE

Single oral moxifloxacin doses of 2000, 500, and 1500 mg/kg were lethal to rats, mice, and cynomolgus monkeys, respectively. The minimum lethal intravenous dose in mice and rats was 100 mg/kg. Toxic signs after administration of a single high dose of moxifloxacin [redacted] ***to these*** animals included CNS and gastrointestinal effects ***such as decreased activity, somnolence, tremor, convulsions, vomiting and diarrhea.***

In the event of acute overdose, the stomach should be emptied. The patient should be carefully observed and given supportive treatment. Adequate hydration must be maintained.

ANIMAL PHARMACOLOGY

There was no evidence of phototoxicity when moxifloxacin was evaluated in hairless mice using simulated sunlight or UVA radiation. In guinea pigs, no skin reactions were observed following moxifloxacin dosing and UVA exposure, but a dose-dependent reddening of the ears was observed at 300 and 500 mg/kg.

Quinolones have been shown to cause arthropathy in immature animals. In studies in juvenile dogs oral doses of moxifloxacin ≥ 30 mg/kg/day (approximately ***1.5 times the maximum recommended human dose based upon systemic exposure*** [redacted] for 28 days resulted in arthropathy. There was no evidence of arthropathy in mature monkeys and rats at oral ***doses*** [redacted] up to ***135 and 500 mg/kg*** [redacted] respectively.

Unlike some other members of the quinolone class, crystalluria was not observed in [redacted] ***6 month repeat dose*** studies in rats and monkeys with moxifloxacin.

Ocular toxicity was not observed in [redacted] ***6 month repeat dose*** studies in rats and monkeys. In beagle dogs, electroretinographic (ERG) changes were observed ***in a 2 week study*** at doses of 60 and 90 mg/kg [redacted] Histopathological changes were observed in the retina from one of four dogs at 90 mg/kg [redacted]

Some quinolones have been reported to have proconvulsant activity that is exacerbated with concomitant use of non-steroidal anti-inflammatory drugs (NSAIDS). Moxifloxacin at an oral dose of 300 mg/kg did not show an increase in acute toxicity or potential for CNS toxicity ***(e.g., seizures)*** in mice when used in combination with NSAIDs ***such as diclofenac, ibuprofen, or fenbufen.***

In animal studies, at [redacted] plasma concentrations about ***5*** [redacted] times the human therapeutic level, a QT-prolonging effect of moxifloxacin was found. Electrophysiological ***in vitro*** studies ***suggested*** [redacted] an inhibition of the rapid activating component of the delayed rectifier potassium current (Ikr) as [redacted] ***an*** underlying mechanism.

SUMMARY AND EVALUATION:

Moxifloxacin is absorbed rapidly from the GI tract of rats, but not quite as quickly from the GI tract of beagle dogs and monkeys following oral administration. The bioavailability of moxifloxacin is highest in beagle dogs (90%) and male rats (80%), but lower in rhesus monkeys (45-57%), minipigs (54%), and female rats (25%). Toxicokinetic studies consistently showed lower serum levels and AUCs in female rats compared to males. Serum protein binding was moderate in all species tested (46-72%). The half life of moxifloxacin in rats is 1-1.5 hr, in rhesus monkeys is about 6.5-7 hr, and in dogs is about 8.6-9 hours. Studies in rats using radiolabeled moxifloxacin demonstrated rapid, extensive tissue distribution following either IV or oral dosing with the exception that moxifloxacin levels in the central nervous system were low. Like many quinolones, moxifloxacin has a high affinity for melanin. After an oral or IV dose of radiolabeled moxifloxacin to rats, little of the drug had been removed from pigmented structures such as the retina, uvea, and meninges 7 days after administration. After oral and intravenous administration of moxifloxacin to male rats, approximately 12-14% of the total dose was excreted into urine within 24 hours. Most of this was unchanged drug (about 75-80%), but 3 other metabolites were also present: an acyl glucuronide, an N-sulfate conjugate, and an oxo-metabolite. The main metabolite in bile from rats was the N-sulfate conjugate (about 60% of the total dose), but the acyl glucuronide (about 7.3% of the total dose), an N-sulfated acyl glucuronide (about 3% of the total dose), and the oxo-metabolite (less than 1% of the total dose) were also detected. In rhesus monkeys, about 56% of a total intravenous dose of radiolabeled moxifloxacin was excreted in feces with about 26% excreted in urine over a 7 day collection period. Most of the urinary excretion occurred during the first 24 hours after dosing. About 7% and 3.3% of the total dose was recovered in the urine and feces (respectively) as unchanged drug. Metabolites identified in the urine of rhesus monkeys (with percentage of the total dose) included: an acyl glucuronide of moxifloxacin (3.6%), the N-sulfate conjugate, a glycolic acid conjugate, a hydroxy-metabolite, an oxo-metabolite, an acyl glucuronide of the oxo-metabolite, a glycolic acid conjugate of the oxo-metabolite, and a monohydroxylated form of the oxo-metabolite (0.5-2.3% each). The main metabolites found in monkey feces were the N-sulfate and the oxo-metabolite (about 30% and 18% of the total dose, respectively). Chiral inversion of the moxifloxacin did not appear to occur in rats or rhesus monkeys; only the S-enantiomer given to the animals intravenously was identified in their urine.

Single oral moxifloxacin doses of 2000 mg/kg and 500 mg/kg were lethal to rats and mice, respectively. The minimum lethal intravenous dose in these rodents was 100 mg/kg. Clinical signs of acute toxicity observed in both mice and rats included decreased motility, staggering gait, labored breathing, narrowed palpebral fissure, soft feces, tremor, and clonic-tonic convulsions. The minimum lethal oral dose of moxifloxacin for cynomolgus monkeys was 1500 mg/kg, although animals receiving single 250 and 750 mg/kg doses also exhibited some clinical signs of toxicity. These included decreased spontaneous activity, somnolence, tremor, and soft stool. Vomiting, tonic convulsion and twitching were observed at $\geq$750 mg/kg, and palpebral ptosis, dyskinesia, mydriasis, and sedation were seen at 1500 mg/kg. Coma occurred prior to death in a monkey at the highest dose level.

Rats dosed orally with 500 mg/kg moxifloxacin for one month demonstrated no drug-related adverse effects other than an initial reduction in body weight gain and soft feces. In a 13 week study with oral dosing, moxifloxacin was associated with a suppression of body weight

gain in male rats at doses ≥100 mg/kg. Rats of both genders treated with ≥500 mg/kg exhibited clinical signs such as piloerection, diarrhea, and salivation. Moxifloxacin was associated with a reduction in hematocrit and hemoglobin in rats from the 750 mg/kg group, particularly males. There was microscopic evidence of slightly increased hematopoietic activity in the high dose group which may be compensatory. Despite elevations in liver transaminases observed in the high dose males, there was no microscopic evidence of liver injury. Cecal dilation, a consequence of the drug's antibiotic effect on the intestinal flora, was seen in all rats treated with moxifloxacin and is a common finding in rodents treated with antimicrobial compounds. The no observed adverse effect level for moxifloxacin after daily oral administration for 13 weeks was 20 mg/kg for males (based upon decrease in body weight gain observed at doses ≥100 mg/kg) and 100 mg/kg for females (based on clinical signs observed in rats of both genders treated with ≥500 mg/kg). In a 6-month oral study in rats, the no observed effect dose was 20 mg/kg for males and 100 mg/kg for females. Male rats at 100 mg/kg demonstrated slight increased serum liver enzymes (ALT and AST) at the end of the study, although there were no microscopic liver changes observed in this group. Males at 500 mg/kg had greater increases in both of these serum enzymes than were observed at 100 mg/kg. A number of males and females in this dose group exhibited degeneration of hepatocytes or diffuse single cell necrosis in their liver tissue with the changes tending to be more pronounced in the males. Several male and female 500 mg/kg rats had multinucleated hepatocytes. Minimal to slight microscopic thyroid changes were observed in some 500 mg/kg male rats, but not females. Body weight gain was reduced in the rats from the 500 mg/kg dose group compared to controls.

In a 4 week study where moxifloxacin was administered intravenously to rats (via the tail vein) at daily doses up to 45 mg/kg, the no observed effect dose was 5 mg/kg for effects at the injection site (reactive inflammation, with necrosis seen in some high dose animals) and 45 mg/kg for systemic effects.

When up to 90 mg/kg of moxifloxacin was given to beagle dogs daily for 4-weeks, the no observed adverse effect level was 10 mg/kg. One female animal in the 90 mg/kg group had to be sacrificed early due to emaciation. Clinical signs of toxicity observed at 90 mg/kg included salivation and occasional vomiting. All of the dogs in this dose group and one in the 30 mg/kg group demonstrated moxifloxacin-induced joint lesions. EKG evaluation suggested slight QT prolongation in the dogs 2 hours after a moxifloxacin dose of 90 mg/kg. Examination of the eyes revealed vacuolization of the subcapsular cortex in the lens in all surviving dogs from the 90 mg/kg group. A 2 week study with oral dosing was performed to specifically investigate oculotoxicity in beagles. The no observed adverse effect for ophthalmic changes (including electroretinography) in this study was 30 mg/kg. Histopathological changes in the retina were observed in the 90 mg/kg group. Other species (monkeys and rats) have not demonstrated histopathological changes in the eye following moxifloxacin dosing. Neither of these species has a tapetum in the eye, unlike the dog. This may be an explanation for the species specificity of the retinal changes. Humans are also a non-tapetal species. A 4 week oral toxicity study with moxifloxacin was performed with even younger dogs (11-13 weeks old). The no observed adverse effect level for 4 weeks of daily oral moxifloxacin doses to these juvenile beagles was 10 mg/kg. Dose-related increases in the incidence and severity of quinolone-induced arthropathy were seen at 30 and 90 mg/kg. Clinical signs of moxifloxacin toxicity such as vomiting and salivation were observed at 90 mg/kg and body weight gain was reduced in this group compared

to controls. Bone marrow toxicity (hypocellularity) was also observed at 90 mg/kg and one female at this dose level had to be sacrificed early due to poor condition.

Cardiac effects of moxifloxacin were investigated in anesthetized dogs. The QT interval was prolonged within 5 minutes following a 30 mg/kg IV bolus dose of BAY 12-8039 (despite tachycardia), but no arrhythmias were seen. QT prolongation was no longer apparent by about 15-30 minutes after drug was given. When a 30 mg/kg IV dose was given over different lengths of time, the QTc rose 20, 33, and 48 msec during the 60, 30, and 15 minute infusions, respectively. The mean maximum plasma concentrations for moxifloxacin immediately following these infusions were 24.3, 45.6, and 63.2 µg/ml. The AUC values were similar regardless of infusion rate. Potassium depletion did not make dogs more sensitive to the QTc prolonging effect of a 30 mg/kg IV moxifloxacin dose given over 30 minutes. Co-administration of [redacted] and moxifloxacin caused a greater increase in QTc prolongation than moxifloxacin alone. The investigators felt that the effect was additive rather than synergistic, but the reviewer is less confident in that conclusion as the experiment did not include dogs given moxifloxacin alone. When an extreme overdose of moxifloxacin (1 mg/kg/min for 60 minutes, then 2 mg/kg/min for 60 minutes, and finally 4 mg/kg/min for 90 minutes) was given to a group of 6 dogs (half received atropine as well), the QT and QTc intervals were increased in a dose-related manner by the moxifloxacin infusions. Increases ranged from about 225 to 500 msec above control. Prolongation was observed beginning with the 1 mg/kg/min infusion and T-wave changes (negative or biphasic) were seen in all dogs. The earliest arrhythmia (AV-nodal extrasystole) was seen in one dog (no atropine) during the 2 mg/kg/min infusion at the 104 minute mark. Changes in P-waves (negative, biphasic) were seen in two of the dogs (with atropine) during the 4 mg/kg/min infusion. The investigator believed that these most likely indicated premature atrial beats, but the report stated that sinus arrest concomitant with an atrial compensatory rhythm could not be excluded. In all but 2 dogs (one with atropine, one without), a variety of rhythm disorders were observed during the 4 mg/kg/min infusion. These included: AV-nodal ectopies, ventricular extrasystoles (doublets and triplets, up to ventricular tachycardia) and bigemini. Two dogs developed torsade de points which reversed spontaneously back to normal rhythm. The 2 dogs that did not develop arrhythmias had QTc intervals of about 690 msec (compared to control QTc of 247-323 msec). Of the 4 dogs that developed arrhythmias, all but one reverted to normal sinus rhythm within 10-20 minutes after stopping the moxifloxacin infusion. The mean plasma levels of moxifloxacin at the end of the 1, 2, and 4 mg/kg/min infusion periods were 50.6, 129, and 265 µg/ml, respectively. These are 11-58 times higher than the anticipated clinical Cmax (4.5 µg/ml) at steady state with daily dosing of 400 mg/day.

A series of *in vitro* experiments were performed to explore the possible mechanism for moxifloxacin's cardiac effects. In cultured AT-1 cells, under appropriate voltage clamp conditions, the only time-dependent outward current in these cells is the rapid component of the delayed rectifier potassium current. The IC_{50} for the reduction of this current was 0.75 ± 0.31 µM for moxifloxacin. CHO DUKX cells stably transfected with the genes for the proteins KvLQT1 and minK were used for other experiments. Together, these transfected proteins form functional potassium channels for the slow component of the delayed rectifier current and this cell line allows specific study of this current. Rubidium flux out through the potassium channels was measured to determine whether these channels were blocked. The cells were loaded with rubidium prior to incubation with test chemicals. Mefenamic acid and/or potassium D-gluconate was used to activate the channels and sparfloxacin and moxifloxacin were included in the system

to see whether they could block the activation. At concentrations up to 30 μM (the highest tested), neither sparfloxacin nor moxifloxacin blocked the activation of rubidium flux from the transfected CHO DUKX cells. This suggests that they are not blockers of the slow component of the delayed rectifier potassium current under the conditions of this experiment. However, the results of these studies were in contrast to an experiment with isolated guinea pig papillary muscle which suggested that moxifloxacin interferes with the slow potassium delayed rectifier current, but did not appear to block the rapid component of this potassium current. It should be noted that the sponsor has presented some preliminary data from additional experiments suggesting that moxifloxacin has the capacity to inhibit the rapid activating component of the delayed rectifier potassium current.

In a 4 week oral moxifloxacin toxicity study in rhesus monkeys, the no effect dose of moxifloxacin 10 mg/kg. A daily dose of 50 mg/kg was associated with minimal clinical signs (occasional instances of animals spitting up dose solution) and no apparent drug-related histopathological changes. The highest dose, 250 mg/kg, was not well tolerated and had to be reduced to 150 mg/kg on day 23. The liver appeared to be a target organ for toxicity with single cell hepatocyte necrosis and increased vacuolation of hepatocytes observed in several monkeys. Additionally, serum liver enzymes were elevated in one male. The investigators theorized that the serous atrophy of the fat marrow in the high dose monkeys was most likely related to the poor condition and reduced nutritional states of these animals. This appears to be a reasonable conclusion. However, there did appear to be a potential for bone marrow toxicity induced by moxifloxacin as hypocellularity was observed in 2 high dose animals. When another 4 week oral study in rhesus monkeys was performed using 150 mg/kg as the highest daily dose, focal hypocellularity in the bone marrow was observed in one male (multiple foci in several bones) and one female (single focus in the scapula) in the high dose group. Histopathological changes in the liver were not seen in this study. In a 13 week oral rhesus monkey study, the no effect level was 15 mg/kg. Vomiting, reduced activity, spasms, and poor nutritional state were observed in monkeys given 135 mg/kg per day. Some animals in this dose group had serous atrophy of bone marrow fat. The differential blood cell counts of this group showed an inversion of the lymphocyte:neutrophil ratio. Such an inversion was also observed in a 4 week IV monkey study when the animals were given 400 mg/monkey per day. The only other drug-related effects in this 4 week IV study were decreased food intake and body weight gain and injection site lesions of a dose-related severity (perivascular and vascular inflammation, edema, fibrin exudation, and focal necrosis). A second 4 week IV monkey study used doses of 5, 15, and 45 mg/kg. Foci with no hematopoiesis were seen in bone marrow smears from some animals that received $\geq$15 mg/kg. Reactive inflammation was seen at the injection sites of all monkeys, but the severity was dose related. In a 26 week oral rhesus monkey study, the no observed adverse effect level was 45 mg/kg. Generalized serous atrophy of the bone marrow (fat portion) was seen in 3 monkeys from the 135 mg/kg group and may be related to reduced nutritional status and poor condition. A hypocellular focus was observed in the bone marrow from a single bone in one 135 mg/kg female, but this observation was not reported for any other bones from this animal or any of the bones from other drug-treated monkeys. The high dose of 135 mg/kg was also associated with reduced food intake, reduced body weight in males, mortality (one female sacrificed in moribund condition) and clinical signs of toxicity in some animals such as spasm, rapid breathing and tendency of the monkeys to assume a lateral position. Inclusions described as "glycogen lakes" were observed in the centriacinar hepatocytes of some monkeys in the 135

mg/kg group. Smaller, but similar inclusions were observed in a few monkeys at 15 and 45 mg/kg as well, but they were comparable to what the pathologist has observed in control monkeys from other studies. Degenerative changes in liver tissue (inflammation or hepatocyte necrosis) were not observed in the 6 month monkey study.

A 0.1% solution of moxifloxacin was not more irritating than 0.9% saline or placebo when administered intravenously to beagle dogs once daily for 10 days at a dose volume of 100 ml. Additionally, a single 2 ml paravasal injection of this moxifloxacin solution was not more irritating to the dogs' tissues than similar injections of saline or placebo. Single intraarterial injections of 0.1% moxifloxacin (approximately 65 ml) were, however, associated with greater tissue irritation than similar injections of placebo or saline. It should be noted that the dogs were kept for a 6-7 day period without treatment prior to sacrifice and microscopic examination of the injection site tissues. Greater differences between the treatment groups may have been observed if the injection sites had not been given time to heal.

In rats, moxifloxacin caused slight paternal toxicity (salivation, changes in feces) at oral doses of 100 and 500 mg/kg, but not 20 mg/kg. A small increase in the percentage of sperm with head-tail separation was seen at the high dose. Slight maternal toxicity (similar to males) was seen only at 500 mg/kg. Estrous cycles of 2 females were lengthened, but both animals were successfully impregnated and had viable litters. Neither male nor female fertility were adversely affected when rats were given doses of moxifloxacin up to 500 mg/kg for 4 or 2 weeks prior to mating, respectively. Early embryonic development of rats was not affected by parental doses of BAY 12-8039 up to 500 mg/kg. Moxifloxacin was not teratogenic to rat fetuses when administered to dams on days 6-17 of pregnancy at oral doses up to 500 mg/kg. The highest dose used in this study, 500 mg/kg, did not cause maternal toxicity, but appeared to have been slightly fetotoxic as it was associated with a greater percentage of litters containing fetuses with non-ossified cervical vertebrae. In an IV rabbit developmental toxicity study, signs of fetotoxicity (reduced placental and fetal weights, retarded ossification) were observed at moxifloxacin doses of 20 mg/kg. There was also a slight increase in the total number of malformations at 20 mg/kg and of combined rib and vertebral malformations. Maternal toxicity (including mortality) was observed at 6.5 and 20 mg/kg. Dose related increases in abortion (secondary to reduced food and water consumption and body weight loss in the does) were also observed at these levels of moxifloxacin. An additional IV developmental toxicity study using a dose of 0.5 mg/kg was conducted in rabbits. Food and water consumption and body weights of the 0.5 mg/kg does were not different from control and necropsy did not reveal any gross drug-related findings. Signs of fetotoxicity (e.g., decreased fetal or placental weights, reduced ossification, decreased fetal survival) were not seen and drug-related malformations or variations were not observed. Oral moxifloxacin doses of 30 and 100 mg/kg given to pregnant cynomolgus monkeys during organogenesis were associated with dose-dependant increases in abortion frequency and maternal toxicity (vomiting, diarrhea). Fetal malformations were not observed at these doses, but fetuses from the 100 mg/kg group tended to be smaller than controls. The 10 mg/kg dose of moxifloxacin was not associated with maternal or fetal toxicity or teratogenicity. In a study of moxifloxacin's pre- and post-natal development effects in rats, females received daily oral doses of drug from day 6 of pregnancy until the end of lactation. Mortality was observed during gestation in the F_0 dams dosed with 500 mg/kg/day of moxifloxacin. Coprophagia was seen occasionally in all moxifloxacin groups and salivation was observed in some dams at 100 and 500 mg/kg. Effects suggestive of fetotoxicity (slight increase in postimplantation loss, decreased

pup birth weight, and increased neonatal mortality) were seen at 500 mg/kg. However, even at 500 mg/kg, there appeared to be no adverse effect on the acquisition of developmental landmarks or the development of hearing and normal reflexes in the pups. Learning, memory, and activity level also appeared normal in the offspring of dams dosed with up to 500 mg/kg of moxifloxacin. There was no impairment of fertility in the F_1 generation at doses up to 500 mg/kg. Pharmacokinetic studies in pregnant and lactating rats indicated that moxifloxacin crosses the placenta and is found in the milk of this species.

Moxifloxacin did not induce reversion of the *Salmonella* TA98, TA100, TA1535, or TA1537, but it should be noted that the concentrations used in this study were very low ($\leq$ 0.16 µg/plate). In *S. typimurium* stain TA102, however, moxifloxacin was associated with an increase in the number of revertants at concentrations of 0.06-0.16 µg/plate, ±S-9. This is consistent with data from other fluoroquinolones which also increased reversion in TA102, but not other bacterial strains. Moxifloxacin did not appear to induce mutations at the HGPRT locus of CHO cells in the presence or absence of S-9. However, in V79 cells, the overall response of moxifloxacin at the HGPRT locus was equivocal. There were increased mutation frequencies in both the absence and presence of S-9, but these tended to be very modest and a dose/response effect was not observed despite the repetition of the assay. Even when all of the data are considered together, a dose-related increase in mutation frequency is not apparent. The reviewer agrees with the investigators that the results of this V-79/HGPRT assay were equivocal. Most of the increased in mutation frequency that were observed in the moxifloxacin-treated cultures were barely greater than the high end of this laboratory's historical negative control range for these cells. The individual positive cultures cannot be discounted completely, but no clear dose/response was observed and the results were not reproducible, although the investigators attempted to do so. Moxifloxacin did not induce UDS in cultured rat hepatocytes at concentrations up to 400 µg/ml, however it is clastogenic in Chinese hamster V79 cells at concentrations $\geq$300 mg/ml regardless of metabolic activation. At oral doses up to 2000 mg/kg, moxifloxacin did not induce an increase in the percentage of mouse bone marrow polychromatic erythrocytes with micronuclei. Moxifloxacin demonstrated no evidence of a dominant lethal effect when a single oral dose of up to 1200 mg/kg was given to male mice. Finally, in what appeared to be a sort of experimental study of initiation and promotion potential in several specific target tissues, moxifloxacin appeared to be neither a tumor initiator nor a tumor promoter at the target sites (liver, kidneys, lungs, mammary glands, urinary bladder, forestomach, bone marrow) when administered to rats orally at a dose of 459 mg/kg. It should be noted, however, that most of the promoters used in this study did not appear to induce much greater incidences of tumors in target organs as did initiators alone, calling into question the sensitivity of this assay in identifying tumor promoters.

Moxifloxacin was not phototoxic to hairless [redacted] mice after a single dose of up to 300 mg/kg followed by exposure to UVA radiation (10 J/cm^2 for 90 minutes) or 7 days of dosing up to 100 mg/kg with the same UVA exposure following each dose of drug. No phototoxic skin reactions were seen in guinea pigs given single 100, 300 or 500 mg/kg doses of moxifloxacin, then irradiated (20 J of UVA/cm^2) approximately 30 minutes after dosing. A repeat dose phototoxicity study with moxifloxacin was also conducted in guinea pigs. The animals were given doses of 100, 300 and 500 mg/kg daily for 7 days, then irradiated (20 J of UVA/cm^2) approximately 30 minutes following each daily dose of drug. Although BAY 12-8039 was not associated with a dorsal skin reaction to UVA, a dose-dependant reddening of the ears was

observed at 300 and 500 mg/kg. This may be indicative of slight phototoxicity to guinea pigs under the conditions of this study. Because moxifloxacin has significant absorption in the UVB portion of the solar spectrum, the sponsor was advised to repeat phototoxicity testing using a light source that includes UVB (e.g., simulated sunlight). Moxifloxacin was not phototoxic when given to hairless mice at a single dose up to 300 mg/kg or after 7 days of dosing up to 100 mg/kg accompanied by simulated sunlight exposure. The positive control, lomefloxacin, induced characteristic phototoxic responses under these conditions. The sponsor also submitted data from some *in vitro* studies with moxifloxacin suggesting that it is more photostable and induces less oxidative DNA damage or DNA strand breakage in the presence of UVA or UVB than ciprofloxacin, BAY y3118, or lomefloxacin. Moxifloxacin was not was photomutagenic at the HGPRT locus of V79-4 cells in the presence of UVA.

Several of the toxic effects observed in animals following moxifloxacin administration are similar to those which have been seen with other quinolones. These include the induction of arthropathy in juvenile dogs, convulsions and other CNS disturbances, and fetotoxicity in the offspring of several species exposed to this drug *in utero*. When moxifloxacin was administered to monkeys at high doses, microscopic liver injury was observed in some animals. In rats, a high dose of moxifloxacin given for a long period of time was also associated with hepatocellular necrosis. Liver changes were not seen in these species when more moderate doses were administered, even for a 6 month dosing period. The potential for moxifloxacin to prolong the QTc was clearly demonstrated in anesthetized dogs. This effect has also been observed in humans. In the dogs, the approved drug, sparfloxacin, appeared to be a more potent prolonger of the QTc than moxifloxacin.

RECOMMENDATIONS: The pharmacologist does not object to the approval of this NDA. The nonclinical data for moxifloxacin are generally similar to other quinolones marketed for clinical use. Though it is less phototoxic than some members of this class, it has been demonstrated to prolong the QTc interval in both dogs and humans (as does sparfloxacin, another marketed fluoroquinolone). The label contains appropriate cautions regarding potential quinolone-related toxicities such as CNS effects, tendon rupture, and juvenile arthropathy. Additionally, the label will contain a warning about QTc prolongation and the potential for cardiac arrhythmias. It will caution against the product's use in patients expected to be particularly vulnerable to adverse cardiac effects.

/S/

Amy L. Ellis, Ph.D.
Pharmacologist, HFD-520

Orig. NDA
cc:
HFD-520
HFD-590
HFD-104
HFD-340

Concurrence Only:
HFD-520/REOsterberg /S/ 12/10/99
HFD-520/LGavrilovich /S/ 12/13/99

HFD-520/Pharm Team Ldr/Osterberg
HFD-590/Pharm Team Ldr/Hastings
HFD-520/Pharm/Ellis
HFD-590/MO/Meyerhoff
HFD-590/MO/Sacks
HFD-590/CSO/Jensen

Histopathology Inventory for NDA #21,085

Study	SBL 95-43	T 4060675	T 1061121	T 6061298	T 9061264
Species	Cynomol.. Monkey	Beagle	Rhesus Monkey	Wistar Rat	Beagle
Adrenals		X*	X*	X*	X*
Aorta		X	X	X	X
Bone Marrow smear	X	X	X	X	X
Bone (femur)	X	X	X	X	X
Brain		X*	X*	X*	X*
Cecum	X	X	X	X	X
Cervix				X	X
Colon	X	X	X	X	X
Duodenum	X	X	X	X	X
Epididymis		X	X	X*	X
Esophagus		X	X	X	X
Eye		X	X	X	X
Fallopian tube		X	X	X	X
Gall bladder		X	X		X
Gross lesions		X	X	X	X
Harderian gland				X	
Heart	X	X*	X*	X*	X*
Ileum	X	X	X	X	X
Injection site					
Jejunum	X	X	X	X	X
Kidneys	X	X*	X*	X*	X*
Lachrymal gland				X	
Larynx				X	
Liver	X	X*	X*	X*	X*
Lungs	X	X*	X*	X	X*
Lymph nodes, cervical					
Lymph nodes mandibular		X	X	X	X
Lymph nodes, mesenteric		X	X	X	X
Mammary Gland				X	
Nasal cavity					
Optic nerves		X	X	X	X
Ovaries		X*	X*	X*	X*
Pancreas		X*	X*	X	X*
Parathyroid		X	X	X	X
Peripheral nerve					
Pharynx					
Pituitary		X*	X*	X	X*
Prostate		X*	X*	X	X*
Rectum	X	X	X	X	X
Salivary gland		X	X	X	X
Sciatic nerve		X	X	X	X
Seminal vesicles			X*		
Skeletal muscle		X	X	X	X
Skin		X	X	X	X
Spinal cord		X	X	X	X
Spleen	X	X*	X*	X*	X*
Sternum	X	X	X	X	X
Stomach	X	X	X	X	X
Testes		X*	X*	X*	X*
Thymus	X	X*	X	X*	X*
Thyroid		X*	X*	X	X*
Tongue		X	X	X	X
Trachea		X	X	X	X
Urinary bladder		X	X	X	X
Uterus		X*	X*	X	X*
Vagina		X	X	X	X
Zymbal gland				X	

* organ weight obtained