their litters. All of these does had markedly reduced food consumption and body weight gain prior to abortion. These findings are commonly observed in pregnant rabbits dosed with anti-infectives. These animals stop eating, most likely due to the antibacterial effects of the drugs in the gut, then they abort.

Body weight gain in the 40, 70, and 100 mg/kg groups was inhibited in a dose-responsive manner. Decreased food intake was also observed in the does from these groups; on several occasions, some animals ate nothing- especially at the highest dose.

Necropsy showed liver discoloration and/or "lobular clarification" in 1 rabbit at 40 mg/kg, 2 at 70 mg/kg, and 1 at 100 mg/kg. One of the 70 mg/kg dams also had an enlarged gall bladder. Another 100 mg/kg rabbit exhibited hydroperitoneum. No significant differences in mean absolute or relative organ weights of the does were observed between treatment groups.

Fetal body weights and placenta weights did not significantly differ between the control group and the surviving litters from gatifloxacin-treated dams. The single surviving live litter in the 100 mg/kg group contained only 4 female fetuses (in contrast to the average of 10 fetuses per litter in the control group). In the 3 surviving litters at 70 mg/kg, the average litter size was around 8, not statistically different from control. No external or skeletal malformations were observed in any of the fetuses.

The concentration of drug in the serum at the 10, 40, and 70 mg/kg dose levels was 0.79-0.96, 3.74-11.50, and 4.64-19.60 μg/ml.

Based upon the results of this study, the investigators recommended that the lowest dose for the pivotal rabbit teratology study be about 10 mg/kg and the highest dose be approximately 40 mg/kg. The 70 and 100 mg/kg doses were considered too high due to the drug-induced reductions in food consumption (and body weight gain) associated with high frequency of abortion. The pivotal rabbit study used 8, 20 and 50 mg/kg doses of gatifloxacin and no drug-related malformations were observed, though some abortions (apparently related to reduced food intake) occurred in each group.

Genetic Toxicology Studies:

BMS-206584: Oral DNA Repair Study in Rats (I), Study No. C931106 (BMS Report No. 910062571)

T. Yasue, H. Kusajima (Kyorin Pharmaceutical Co., Central Research Laboratories, Tochigi, Japan)

Report dated 1/94, not GLP

Vol. 33, pp. 94-105

Animals: Male F344/DuCrj rats, 10-12 weeks old, 210-272 g, 3 animals in negative control group and 4 in other groups

Diet: not specified

Drug Dose and Route of Administration: Gatifloxacin (Lot No. G15531) was suspended in 0.3% carboxymethyl cellulose (also used as the negative control) and given at a dose of 1000 mg/kg. The positive control, 2-acetylaminofluorene (AAF), was dissolved in polyethylene glycol and given at a dose of 100 mg/kg. Test substances were administered at a dose volume of 10 mg/kg via oral gavage.

Length of Study and Method: Animals were anesthetized 4 hours after test substances were given, blood samples were drawn for the measurement of serum gatifloxacin, and their livers were perfused *in situ* with collagenase solution. Isolated hepatocytes were suspended in William's medium E with 10% fetal bovine serum and incubated at 37° for 60-90 minutes to allow cells to adhere to cover slips. Cells that adhered to the cover slips were incubated with medium containing 10 μCi/ml of ^{3}H-thymidine for 4 hours. After washing with fresh medium, cells were cultured for another 14-16 hours in fresh medium containing 0.25 mM of non-radiolabeled thymidine. Next, cultured hepatocytes were exposed to hypotonic conditions before fixing onto cover slips. They were mounted onto slides, coated with autoradiographic emulsion, and developed. Slides were stained with hematoxylin before microscopic examination. The number of silver grains in 100 cells per rat (4 cover slips) were counted.

Results: The numbers of nuclear, cytoplasmic, and net (nuclear minus cytoplasmic) silver grains per rat did not differ between the negative control and 1000 mg/kg gatifloxacin groups. In contrast, unscheduled DNA synthesis was observed in all of the rats treated with AAF. The mean net silver grains per nucleus in the control, gatifloxacin, and AAF rats was -2.8 ± 2.0, -4.0 ± 0.5, and 12.4 ± 3.1, respectively.

Unscheduled DNA synthesis was not induced in rat hepatocytes after the animals were given an oral 1000 mg/kg dose of gatifloxacin. The mean serum level of the drug in these rats was 33.9 ± 9.9 μg/ml and the estimated concentration in the liver (based upon tissue distribution studies) was 162.7 μg/g.

BMS-206584: Oral DNA Repair Study in Rats (II), Study No. 18250-0-494 (BMS Report No. 910060757)

[redacted]

Report dated: 4/18/97, U.S. GLP

Vol. 33, pp. 106-178

Animals: Male and female Sprague-Dawley (Hsd:SD) rats, 7 weeks old, 6/sex per dose group

Diet: PMI Certified Rodent Diet 5002 and water were available *ad libitum*.

Drug Dose and Route of Administration: Gatifloxacin (Batch No. G6X5311) was suspended in 0.3% carboxymethyl cellulose (also used as the negative control) and given via oral gavage at doses of 200, 600, and 2000 mg/kg at dose volumes of up to 20 mg/kg. The positive control, dimethylnitrosamine (DMN), was dissolved in sterile deionized water and given at doses of 10

and 15 mg/kg via intraperitoneal injection (for the 2-3 hour time point and the 15-16 hour time point, respectively.

Length of Study and Method: Three rats/sex/dose group were anesthetized 2-3 or 15-16 hours after test substances were given, and their livers were perfused *in situ* with a series of culture media, salt solutions (without Ca or Mg) and collagenase. Isolated hepatocytes were suspended in William's medium E (with 2 mM l-glutamine, 100 µg/ml streptomycin sulfate, 150 µg/ml gentamycin, and 10% fetal bovine serum) and incubated at 35-37° for approximately 2 hours to allow cells to adhere to cover slips (or to dishes, for the assessment of attachment efficiency). Cells that adhered to the cover slips were incubated with medium containing 10 µCi/ml of ^{3}H-thymidine for 4 hours. After washing with fresh medium, cells were cultured for approximately 18 hours in fresh medium containing 0.25 mM of non-radiolabeled thymidine. Next, cultured hepatocytes were exposed to hypotonic conditions before fixing onto cover slips. They were mounted onto slides, coated with autoradiographic emulsion, and stored in a light proof box for 8-10 days before developing. Slides were stained with hematoxylin and eosin before microscopic examination by a blinded technician. The number of silver grains in 3 replicate cultures per rat were counted, at least 100 cells per animal. Cells undergoing DNA replication (as opposed to repair) were excluded. Cells with nuclei containing 5 or more net grains were considered to be undergoing UDS. No more than 10% of the cells in the vehicle control group should contain 5 or more net nuclear grains in order for the assay to be valid.

Results: Bloating of the intestinal tract was observed at the time of perfusion in the mid and high dose males at both time points and in the female animals from these dose groups at the 15-16 hour time point.

The attachment efficiencies (40-125%) and viability of the attached cells (74-98% for both genders at both time points).

The numbers of net (nuclear minus cytoplasmic) silver grains per rat did not differ between the negative control and gatifloxacin groups, regardless of gender or time point. In contrast, unscheduled DNA synthesis was observed in all of the rats treated with DMN.

UDS in Rat Hepatocytes After Oral Administration of Gatifloxacin

Treatment Groups	Mean Net Nuclear Grains (NGs)		% Cells with >5 Mean Net NGs	
	Males	Females	Males	Females
2-3 Hour Time Point				
Vehicle	-1.05	-1.42	1.56	0.44
200 mg/kg Gatifloxacin	-1.26	-1.16	0.43	0.00
600 mg/kg Gatifloxacin	-1.29	-0.93	0.44	0.44
2000 mg/kg Gatifloxacin	-1.27	-1.29	0.67	0.89
10 mg/kg DMN	6.02	11.56	62.0	92.22
15-16 Hour Time Point				
Vehicle	-0.88	-0.94	1.56	0.57
200 mg/kg Gatifloxacin	-0.75	-0.62	0.89	1.00
600 mg/kg Gatifloxacin	-1.05	-0.71	0.00	0.67
2000 mg/kg Gatifloxacin	-0.92	-0.72	0.91	0.44
15 mg/kg DMN	6.51	5.97	61.67	59.56

Unscheduled DNA synthesis was not induced in rat hepatocytes after the animals were given oral doses of gatifloxacin up to 2000 mg/kg.

Special Toxicity Studies:

BMS-206584: Single-Dose Intravenous Phototoxicity Study in Mice, Study No. C91EM13 (BMS Report No. 910070391)

K. Tsuru, M. Nagata, H. Ogata, Y. Kuninishi (Kyorin Pharmaceutical Co., Tochigi, Japan)

Report dated: 1/31/92, Japanese GLP

Vol. 44, pp. 123-147

Animals: Male Crj:CD-1 (ICR) mice, 6 weeks old, 26.5-33.9 g, 5 per gatifloxacin dose group, 4 in the positive control group, housed 4-5 per cage on woodchip bedding, dorsum shaved one day prior to dosing and UV exposure

Diet: CE-2 diet [redacted] and filtered well water were provided *ad libitum*.

Drug Dose and Route of Administration: Gatifloxacin (Lot No. S080070) was dissolved in physiological saline and given via IV injection at doses of 15, 30, and 60 mg/kg (20 ml/kg dose volume). [redacted] was suspended in 0.3% carboxymethyl cellulose and given orally at 25 mg/kg in a volume of 10 ml/kg.

Length and Conduct of Study: Animals were given a single dose of drug and a half hour later they were irradiated with UVA light [redacted] at a constant intensity of 400 $\mu W/cm^2$ for 3 hours (total dose of 4.3 J/cm^2). During irradiation, the right side of the dorsum was protected [redacted] The mice were examined for phototoxic reactions and the right and left sides of the dorsum compared immediately following UVA exposure, then 24 hours, 48 hours, and 7 days later.

Results: Phototoxic reactions were not observed in the mice given single IV doses of gatifloxacin up to 60 mg/kg and exposed to UVA radiation. Three of the 4 mice which received [redacted] demonstrated marked edema (with or without accompanying erythema) 24 and 48 hours after treatment. Seven days after treatment, edema and erythema were still present and crusting and exfoliation of the UVA exposed sites was observed. It is not clear why the forth positive control mouse did not exhibit a phototoxic reaction.

Gatifloxacin did not induce phototoxicity in the presence of UVA radiation under the conditions of this study, but the compound has its peak absorption in the UVB portion of the solar spectrum. Thus, the sponsor was advised to repeat phototoxicity testing using a light source that includes UVB (e.g., simulated sunlight).

BMS-206584: Single-Dose Oral Phototoxicity Study in Guinea Pigs, Study No. A95VG05 (BMS Report No. 910070392)

Y. Kuninishi, A. Omodera, Y. Nomoto (Kyorin Pharmaceutical Co., Tochigi, Japan)

Report dated: 9/28/95, Japanese GLP

Vol. 46, pp. 1-18

Animals: Male Hartley guinea pigs, 6 weeks old, 394-456 g, dorsum shaved one day prior to dosing and UV exposure

Diet: LCR4 diet [redacted] and filtered tap water were provided *ad libitum*.

Drug Dose and Route of Administration: Gatifloxacin (Lot No. G3X5321) was suspended in 0.3% carboxymethyl cellulose and administered orally at a dose volume of 10 ml/kg. The doses used were 0 (vehicle control), 100 and 200 mg/kg.

Length and Conduct of Study: Animals were given a single oral dose of drug and a half hour later they were irradiated with UVA light [redacted] at a constant intensity of [redacted] During irradiation, the right side of the dorsum was protected [redacted] The guinea pigs were examined for phototoxic reactions and the right and left sides of the dorsum compared immediately following UVA exposure and 24 and 48 hours later.

Results: Gatifloxacin did not induce a phototoxic reaction in the guinea pigs in the presence of UVA radiation under the conditions of this study after a single dose of up to 200 mg/kg. Slight erythema was observed in 1 control animal and 1 animal from the 200 mg/kg group. No skin reactions were seen in any animal at the 24 and 48 hour observation times.

BMS-206584: Photosensitivity Potential in Guinea Pigs (BMS Report No. 910062570)

I. Nakagawa, S. Isogai, Y. Kuninishi, Y. Nomoto (Kyorin Pharmaceutical Co., Japan)

Report dated: 5/11/95, not GLP

Vol. 46, pp. 36-56

Animals: Male Hartley guinea pigs, 6-7 weeks old, 324-424 g, 5 per dose group, a 2 X 4 cm area of the neck was clipped one day prior to the first dose and UV exposure and on the day prior to challenge

Diet: CG-7 diet [redacted] and filtered tap and well water were provided *ad libitum*.

Drug Dose and Route of Administration: Gatifloxacin (Lot No. G3X5321) doses of 50 and 100 mg/kg/day were used to sensitize the guinea pigs. The drug was suspended in 0.3% carboxymethyl cellulose and was administered orally at a dose volume of 10 ml/kg to sensitize the animals. Groups that were pre-treated with cyclophosphamide received one dose of this drug 3 days before the start of the sensitization. Cyclophosphamide (CPA) was dissolved in normal saline and administered intraperitoneally at a dose of 200 mg/kg with a dose volume of 5 ml/kg.

Treatment groups were as follows:

1. Sensitized with vehicle (0.3% CMC), challenged with vehicle
2. Pretreated with CPA, sensitized with vehicle, challenged with vehicle
3. Sensitized with vehicle, challenged with gatifloxacin (dose unclear)
4. Pretreated with CPA, sensitized with vehicle, challenged with gatifloxacin (dose unclear)
5. Sensitized and challenged with 50 mg/kg gatifloxacin
6. Pretreated with CPA, sensitized and challenged with 50 mg/kg gatifloxacin
7. Sensitized and challenged with 100 mg/kg gatifloxacin
8. Pretreated with CPA, sensitized and challenged with 100 mg/kg gatifloxacin

For sensitization, guinea pigs were dosed with gatifloxacin or vehicle (following pretreatment with cyclophosphamide 3 days earlier, as appropriate). Thirty minutes after administration, animals were irradiated [redacted] with 3000 $\mu w/cm^2$ of UVA for 170 minutes for a total radiation dose of 30 J/cm^2. This was repeated once daily for 5 days. The challenge occurred 17 days after the final sensitization exposure. Following the challenge dose of vehicle or drug, guinea pigs were irradiated as above if they were not pretreated with CPA. During the challenge radiation exposure, half of the clipped area was covered with [redacted] comparison. The animals that were pretreated with CPA received half of the radiation dose (e.g., 85 minutes of exposure to the same radiation source) because the purpose of this pretreatment is to make the guinea pigs more susceptible to photosensitization. The clipped irradiated area was compared to the [redacted] area immediately after irradiation, then 24 and 48 hours after.

Results: Food consumption was reduced in the 50 mg/kg gatifloxacin group (± CPA) starting 2 days after the start of sensitization. The animals were eating at control levels within 6 days after the end of the sensitization period. Two guinea pigs receiving 100 mg/kg of gatifloxacin died 5-6 days after sensitization was complete- one was from CPA pretreatment group and the other had not received the CPA pretreatment. Abdominal fullness and diarrhea were observed in gatifloxacin-treated guinea pigs.

Gatifloxacin with UVA radiation did not induce photosensitization in guinea pigs under the conditions of this study, regardless of pretreatment with cyclophosphamide. Erythema scores did not exceed those observed in the control groups.

BMS-206584: Photosensitization Study in Guinea Pigs (I), Study No. C94EG17 (BMS Report No. 910072025)

I. Nakagawa, S. Isogai, Y. Kuninishi, Y. Nomoto (Kyorin Pharmaceutical Co., Tochigi, Japan)

Report dated: 5/16/95, Japanese GLP

Vol. 46, pp. 57-75

Animals: Male Hartley guinea pigs, 6 weeks old, 324-382 g, 5 per dose group, a 2 X 4 cm area of the neck was clipped one day prior to the first dose and UV exposure and on the day prior to challenge

Diet: CG-7 diet [redacted] and filtered tap and well water were provided *ad libitum*.

Drug Dose and Route of Administration: Gatifloxacin (Lot No. G3X5321) at a dose of 100 mg/kg/day was used to sensitize the guinea pigs. The drug was suspended in 0.3% carboxymethyl cellulose and was administered orally at a dose volume of 10 ml/kg to sensitize the animals. Groups that were pre-treated with cyclophosphamide received one dose of this drug 3 days before the start of the sensitization. Cyclophosphamide (CPA) was dissolved in normal saline and administered intraperitoneally at a dose of 200 mg/kg with a dose volume of 5 ml/kg.

Treatment groups were as follows:

1. Sensitized with vehicle (0.3% CMC), challenged with vehicle
2. Pretreated with CPA, sensitized with vehicle, challenged with vehicle
3. Sensitized with vehicle, challenged with 100 mg/kg gatifloxacin
4. Pretreated with CPA, sensitized with vehicle, challenged with 100 mg/kg gatifloxacin
5. Sensitized and challenged with 100 mg/kg gatifloxacin
6. Pretreated with CPA, sensitized and challenged with 100 mg/kg gatifloxacin

The procedures for sensitization, challenge, and observation were the same as for BMS Report No. 910062570 (directly above).

Results: This seems to be a final report of the same study that was discussed above with the exception that no mention is made of a 50 mg/kg gatifloxacin group. The data from both this report and that one are strikingly similar.

Two guinea pigs sensitized with gatifloxacin died 5-6 days after the end of the sensitization period (one treated with cyclophosphamide and the other not). Abdominal distension and diarrhea were observed during treatment with gatifloxacin and resolved prior to the challenge.

Gatifloxacin with UVA radiation did not induce photosensitization in guinea pigs under the conditions of this study, regardless of pretreatment with cyclophosphamide. Erythema scores did not exceed those observed in the control groups.

BMS-206584: Photosensitization Study in Guinea Pigs (II), Study No. C95EG01 (BMS Report No. 910072024)

I. Nakagawa, S. Isogai, Y. Kuninishi, Y. Nomoto (Kyorin Pharmaceutical Co., Tochigi, Japan)

Report dated: 5/16/95, Japanese GLP

Vol. 46, pp. 76-93

Animals: Male Hartley guinea pigs, 6 weeks old, 349-424 g, 5 per dose group, a 2 X 4 cm area of the neck was clipped one day prior to the first dose and UV exposure and on the day prior to challenge

Diet: CG-7 diet [redacted] filtered tap and well water were provided *ad libitum*.

Drug Dose and Route of Administration: Gatifloxacin (Lot No. G3X5321) at a dose of 50 mg/kg/day was used to sensitize the guinea pigs. The drug was suspended in 0.3% carboxymethyl cellulose and was administered orally at a dose volume of 10 ml/kg to sensitize the animals. Groups that were pre-treated with cyclophosphamide received one dose of this drug 3 days before the start of the sensitization. Cyclophosphamide (CPA) was dissolved in normal saline and administered intraperitoneally at a dose of 200 mg/kg with a dose volume of 5 ml/kg. Treatment groups were as follows:

1. Sensitized with vehicle (0.3% CMC), challenged with vehicle
2. Pretreated with CPA, sensitized with vehicle, challenged with vehicle
3. Sensitized with vehicle, challenged with 50 mg/kg gatifloxacin
4. Pretreated with CPA, sensitized with vehicle, challenged with 50 mg/kg gatifloxacin
5. Sensitized and challenged with 50 mg/kg gatifloxacin
6. Pretreated with CPA, sensitized and challenged with 50 mg/kg gatifloxacin

The procedures for sensitization, challenge, and observation were the same as for BMS Report No. 910062570 (above).

Results: This seems to be a final report of the same study that was discussed above with the exception that no mention is made of a 100 mg/kg gatifloxacin group. The data from both this report and that one are strikingly similar. The original developer of the drug product appears to have given BMS a report summarizing the results of both gatifloxacin studies (one using 50 mg/kg and the other using 100 mg/kg) as well as final reports of the individual studies using each dose.

No mortality occurred during the study. Reduced food consumption was noted in some animals beginning 2 days after the initiation of sensitization and resolving within 5 days after the sensitization period was over.

Gatifloxacin with UVA radiation did not induce photosensitization in guinea pigs under the conditions of this study, regardless of pretreatment with cyclophosphamide. Erythema scores did not exceed those observed in the control groups.

BMS-206584: Effects of Intravenous Infusion on Pulmonary Function in Anesthetized Guinea Pigs, Study No. OF-PH 346 (BMS Report No. 910070393)

[redacted]

Report dated: 3/3/98

Vol. 46, pp. 225-244

Summary: Male guinea pigs (230-300g, 10 per treatment group) were anesthetized with ketamine/xylazine. Tracheal and esophageal cannulas were inserted for measurement of respiration. Transpulmonary pressure was measured with a differential transducer attached to the esophageal cannula. Air flow was measured with a pressure transduced connected to the tracheal cannula. The guinea pigs received a 30 minute infusion of vehicle (0.9% saline), gatifloxacin or ciprofloxacin at a rate of 1 mg/kg/min (0.5 ml/min) into the jugular vein following a 15-30 minute equilibration of the anesthetized, cannulated animals. Respiratory measurements continued 30 minutes after the end of infusion. Histamine (0.215-4.64 μg/kg) given as a bolus infusion was used as a positive control.

Neither gatifloxacin nor ciprofloxacin caused any change in respiratory parameters during infusion. Gatifloxacin was associated with a very small increase in respiratory frequency and a slight decrease in tidal volume 20-30 minutes after the end of infusion compared to vehicle. No significant changes in compliance were observed with gatifloxacin- an increase in compliance observed in the ciprofloxacin group was not statistically significant compared to control. Neither drug was associated with a change in airway resistance. Histamine caused the expected respiratory changes (decreased tidal volume with increased resistance, compliance and respiratory frequency).

Gatifloxacin was not associated with biologically significant changes in respiration when infused IV to guinea pigs at a rate of 1 mg/kg/min for 30 minutes.

BMS-206584: Local Tolerability After Single Intravenous, Intraarterial, and Paravenous Injection in the Rabbit, Study No. FO-TP2004/96 (BMS Report No. 910062572)

[redacted]

Report dated: 6/12/96

Vol. 47, pp. 141-171

Summary: Groups of 6 female New Zealand White rabbits received intravenous, intraarterial, and paravenous injections of gatifloxacin HCl (Batch No. 0016918) or gatifloxacin citrate (Batch No. 0016919) into the right ears and received similar injections of either the HCl or citric acid vehicles into the left ears. Half of the animals were sacrificed 24 hours after the injections and the rest were sacrificed 72 hours after the injections. The tissues at the injection sites were excised and examined microscopically after appropriate preparation and staining.

Intraarterial injection of either gatifloxacin batch caused vocalization and severe defensive reactions, so only one animal per gatifloxacin formulation was injected by this route. Paravenous injection caused less severe defensive reactions in 5/6 receiving gatifloxacin HCl, 4/6 receiving HCl placebo, 4/6 receiving gatifloxacin citrate, and 3/6 receiving citric acid placebo. Intravenous administration caused a defensive reaction in only 1/6 rabbits receiving gatifloxacin HCl, none receiving the HCl placebo or gatifloxacin citrate, and 3/6 receiving citric acid placebo.

The only gross or microscopic lesions at the injection sites were related to mechanical trauma secondary to the injection procedures.

Intravenous administration of either gatifloxacin formulation was well tolerated by the rabbits, but paravenous or intraarterial administration both appeared painful to the animals despite the lack of microscopic changes at the sites of injection.

Toxicity Studies with Impurities of BMS-206584:

BMS-206584 Impurities: Single-Dose Oral Toxicity Study in Rats (I), Study No. A96AR16 (BMS Report No. 910070394)

T. Tsuchiya, M. Yamasaki, Y. Dewa, H. Tanase, S. Iwasaki, K. Tsuru (Kyorin Pharmaceutical Co., Tochigi, Japan)

Report dated: 12/17/96, Japanese GLP

Vol. 48, pp. 1-82

Animals: Male and female Wistar rats, 6 weeks old, 5/sex per dose group

Diet: CRF-1 solid diet [redacted] and filtered tap water were available *ad libitum*. Rats were fasted from the evening prior to dosing until 3 hours after test substance was administered.

Drug Dose and Route of Administration: Single oral doses (500, 1000, and 2000 mg/kg) of an [redacted] gatifloxacin impurity (Lot No. G665316) or a [redacted] gatifloxacin impurity (Lot No. G645326) were given to rats as suspensions in distilled water. The respective dose volumes for each dose level were 5, 10, and 20 ml/kg.

Length of Study: Single oral gavage doses of the test compounds were administered and the animals were observed for the next 14 days.

Results: In the [redacted] mortality on day 1 was 1/5, 4/5, and 5/5 for males and 2/5, 5/5, and 5/5 for females for the 500, 1000, and 2000 mg/kg dose groups, respectively. No other deaths occurred. It should be noted that many of the deaths appeared to be due to dosing accident, as hemorrhage and fluid were frequently found in the lung. Clinical signs observed in the rats dying on day 1 included salivation, oral and/or nasal hemorrhage, tremor, and

convulsions. Deaths occurred within 30 minutes to 6 hours after dosing. Some of the rats that survived also demonstrated these signs.

In the 500 mg/kg 2-methylpiperazine group, 1/5 male rats died on day 1. This animal experienced a tonic/clonic convulsion, but its death may have been secondary to a dosing accident. Clinical signs in the surviving rats included ptosis, convulsions, and salivation. No other mortality was observed for the rest of the study.

The study will be repeated since the data from this one are confounded by the number of animals dying due to dosing accidents.

BMS-206584 Impurities: Single-Dose Oral Toxicity Study in Rats (II), Study No. A96AR25 (BMS Report No. 910070397)

T. Tsuchiya, M. Yamasaki, Y. Dewa, H. Tanase, S. Iwasaki, K. Tsuru (Kyorin Pharmaceutical Co., Tochigi, Japan)

Report dated: 2/17/97, Japanese GLP

Vol. 48, pp. 83-184

Animals: Male and female Wistar rats, 6 weeks old, 5/sex per dose group

Diet: CRF-1 solid diet [redacted] and filtered tap water were available *ad libitum*. Rats were fasted from the evening prior to dosing until 3 hours after test substance was administered.

Drug Dose and Route of Administration: Single oral doses (125, 250, 500, and 1000 mg/kg) of an N-methyl gatifloxacin impurity (Lot No. G665316) or a 2-methylpiperazine gatifloxacin impurity (Lot No. K6Y5316) were given to rats as suspensions in distilled water. The dose volume was 10 ml/kg.

Length of Study: Single oral gavage doses of the test compounds were administered and the animals were observed for the next 14 days.

Results: In the N-methyl group, mortality on day 1 was 0/5 3/5, 4/5, and 5/5 for males and 0/5, 0/5, 3/5, and 5/5 for females for the 125, 250, 500, and 1000 mg/kg dose groups, respectively. No other deaths occurred. Clinical signs observed in both male and female rats dying on day 1 included salivation, nasal hemorrhage, decreased spontaneous activity, tremor, and convulsions. Deaths occurred within 30 minutes to 2 hours after dosing. The male rat that survived at 500 mg/kg also demonstrated many of these signs, but the 2 male survivors at 250 mg/kg did not demonstrate any clinical signs. The 2 surviving females at 500 mg/kg showed only tremor and decreased spontaneous activity for up to 2 hours after dosing and the females in the 125 and 250 mg/kg dose groups did not exhibit any clinical signs. At necropsy, none of the survivors had any abnormal gross findings. Most of the animals that died on day 1 exhibited lung hemorrhage and several in the 250 and 500 mg/kg group had foamy or white solution in the lung. The investigators believed that the fluid in the lung was the test substance, but they attributed the

finding not to dosing accidents, but to inhalation of test substance due to dyspnea from the convulsions.

In the 2-methylpiperazine group, there was no mortality. Clinical signs in males at 500 and 1000 mg/kg included tremor, convulsions, and salivation. No clinical signs were observed in any of the females. None of the animals had any abnormal gross findings at necropsy.

The N-methyl and 2-methylpiperazine forms of gatifloxacin appear more toxic than the parent compound as the acute lethal dose in the rat for the parent was more than 2000 mg/kg for both genders and convulsions were not observed at this dose level. Mortality in rats was observed at 250 mg/kg of N-methyl gatifloxacin. The 2-methylpiperazine gatifloxacin was associated with convulsions at doses $\geq$ 500 mg/kg.

BMS-206584 Impurities: Two-Week Oral Toxicity Study in Rats, Study No. A97SR03 (BMS Report No. 910070422)

M. Kasai, Y. Masumoto, C. Matsumoto, Y. Kaneko, K. Takizawa, H. Ogata, Y. Kuninishi, K. Mashiko, Y. Okumura, H. Tanase, A. Ikeda, Y. Sato, H. Takagi, S. Iwasaki (Kyorin Pharmaceutical Co., Tochigi, Japan)

Report dated: 6/5/98, Japanese GLP

Vol. 49, pp. 1-256

Animals: Male and female Wistar rats, 6 weeks old, 10/sex per dose group

Diet: CRF-1 solid diet [redacted] and filtered tap water were available *ad libitum*.

Drug Dose and Route of Administration: Gatifloxacin (Lot No. G745351) was given to rats by itself, or after being spiked with an N-methyl impurity (Lot No. G765316) and a 2-methylpiperazine impurity (Lot No. K765326) at 1% each. The dose groups were 0 (vehicle control), 90 and 270 mg/kg of gatifloxacin and 90 and 270 mg/kg of gatifloxacin plus impurities. The test compounds were suspended in 0.3% carboxymethyl cellulose and given in a dose volume of 10 ml/kg once daily for 14-16 consecutive days.

Length and Conduct of Study: Rats received test compounds for 14-16 days and were sacrificed on the day following their last dose of drug. Body weights and food consumption were determined twice weekly. Blood samples were taken at necropsy for hematology and clinical chemistry measurements. Femoral bone marrow was collected from the control and high dose groups for bone marrow smears to be examined microscopically. Urine samples were collected on days 10-11 from 6 rats/sex in each group. Ophthalmoscopic examination was performed on 6 rats/sex in each group on day 8 or 9. Microscopic examination was performed on all harvested tissues from the control and high dose groups. Tissues with gross lesions, and the liver, kidneys, and cecum from all dose groups were examined microscopically.

Results: Abnormal clinical signs were not observed in any of the rats. No mortality occurred during the dosing period. Drug-related changes in body weight gain and food consumption were not observed.

Biologically significant changes were not observed in hematologic (including bone marrow smears) or serum chemistry parameters. Urinalysis revealed an increased potassium level in males from either high dose group (with or without impurities). Ophthalmic examination did not show any drug-related changes.

Cecal weights were increased compared to control in all groups of drug-treated rats. Small decreases in the mean absolute and relative heart and liver weights were seen in gatifloxacin-treated rats. No drug-related gross changes were observed at necropsy other than cecal enlargement. Microscopic examination revealed a slight increase in fatty droplets in hepatocytes in 3 females in the high dose gatifloxacin group and in 5 females and 1 male from the high dose gatifloxacin plus impurities group. No other microscopic changes related to gatifloxacin treatment (with or without impurities) were seen. Histopathological changes were not observed in the cecum, pancreas, or articular cartilage in any group of rats.

Addition of N-methyl and 2-methylpiperazine impurities to 1% each did not increase to toxicity of gatifloxacin to rats at oral doses of up to 270 mg/kg for 14-16 days.

BMS-206584 Impurities: Ames Reverse-Mutation Study in Salmonella and Escherichia coli, Study No. A96MB10 (BMS Report No. 910070395)

Y. Kasahara, K. Mashiko, N. Kusumoto (Kyorin Pharmaceutical Co., Central Research Laboratories, Tochigi, Japan)

Report dated 9/4/96, Japanese GLP

Vol. 49, pp. 257-301

Strains Used: *Salmonella typhimurium* TA98, TA100, TA1535, TA1537 and *E. coli* WP2*uvr*A-

Method: The plate incorporation method with a preincubation step was used to investigate gatifloxacin's mutagenic potential in bacteria. Bacteria were combined with test substances ± S-9 and incubated for 20 minutes at 37°C with shaking. Next, molten overlay agar (0.6% agar with 0.5% NaCl supplemented with either 0.05 mM histidine and biotin or 0.05 mM tryptophan depending on bacteria used) was added to the bacterial culture and the suspension was plated on minimal agar (Vogel-Bonner minimal medium with 1.5% agar and 2% glucose). Duplicate plates were incubated for approximately 48 hours at 37°C before revertants were counted. S-9 mix derived from phenobarbital- and 5,6-benzoflavone-induced male Sprague-Dawley rats was added to the overlay agar as applicable. Positive controls in the absence of S-9 were 2-(2-furyl)-3-(5-nitro-2-furyl) acrylamide (10 ng/plate for TA100 and WP2*uvr*A-; 100 ng/plate for TA98), sodium azide (500 ng/plate for TA1535), and 9-aminoacridine hydrochloride (80 µg/plate for TA1537). The positive control in the presence of S-9 was 2-aminoanthracene (500 ng/plate for TA98; 1000 ng/plate for TA100, 2000 ng/plate for TA1535 and TA1537, and 10000 ng/plate for WP2*uvr*A-). A dose range finding study using TA100 was conducted at concentrations of test article up to 5 mg/plate to establish the cytotoxic limits for gatifloxacin in the presence and

absence of S-9. Preliminary mutagenicity studies were conducted for each impurity to choose the amounts per plate to use in the "main" mutagenicity study. The amounts of drug per plate used in the preliminary studies (± S-9) were 0.05-100 ng and 0.1-500 ng for the N-methyl and 2-methylpiperazine forms of gatifloxacin, respectively. In the main study, the amounts of N-methyl gatifloxacin per plate used for the *Salmonella* strains were 0.39, 0.78, 1.56, 3.13, 6.25, 12.5, 25, and 50 ng and the amounts for *E. coli* WP2*uvr*A- were 6.25, 12.5, 25, 50, 100, 200, 400, and 800 ng (both ± S-9). In the main study with 2-methylpiperazine gatifloxacin, the amounts per plate used for the *Salmonella* strains were 3.9, 7.8, 15.6, 31.3, 62.5, 125, 250, and 500 ng and the amounts for *E. coli* WP2*uvr*A- were 31.3, 62.5, 125, 250, 500, 1000, 2000, and 4000 ng (both ± S-9). The highest concentrations of gatifloxacin used in the "main" study caused reductions in the number of revertants on selective agar and a diminution of the bacterial lawn. The solvent for the N-methyl (Lot No. G645316) and 2-methylpiperazine (Lot No. G645326) forms of gatifloxacin and for the positive control substances was DMSO.

Results: Acceptable numbers of bacterial revertants were observed on control plates in all strains for both the preliminary and main mutagenicity studies and positive controls performed adequately. No compound-related increases in the number of bacterial revertants compared to control was observed in any strain of bacteria at any dose level of either the N-methyl or 2-methylpiperazine gatifloxacin impurity regardless of metabolic activation.

Under the conditions of this study, neither gatifloxacin impurity induced reversion of the *Salmonella* or *E. coli* strains tested. However, due to the cytotoxicity of the gatifloxacin impurities (which appear to have antimicrobial activity), the concentrations used in this study were very low (generally less than 0.5 µg/plate).

BMS-206584 Impurities: Cytogenetics Study in Chinese Hamster Lung Cells, Study No. A96MC11 (BMS Report No. 910070396)

N. Kusumoto, K. Mashiko, Y. Kasahara (Kyorin Pharmaceutical Co., Central Research Laboratories, Tochigi, Japan)

Report dated 10/25/96, Japanese [redacted]

Vol. 49, pp. 302-343

Method: Chinese hamster lung (CHL/IU) cells (cultured in Eagle's minimal essential medium supplemented with glutamine, $NaHCO_3$, and calf serum) were incubated with test substances in the absence of metabolic activation for 24 or 48 hours at 37°C. Gatifloxacin impurities were tested separately. The concentrations of the N-methyl (Lot No. G645316) and 2-methylpiperazine (Lot No. G645326) forms of gatifloxacin used for the 24 hour incubation were 90, 180, and 360 µg/ml and 88, 175, and 350 µg/ml, respectively. For the 48 hour incubations, the concentrations of the N-methyl and 2-methylpiperazine impurities were 75, 150 , and 300 µg/ml and 44, 88, 175 µg/ml, respectively. In the presence of metabolic activation, cells were incubated with S-9 and test substances for 6 hours (there were also cells incubated with test substances in the absence of S-9 for the same length of time), then incubated further in drug-free medium for 18 hours longer. The concentrations of either gatifloxacin impurity used in the S-9

experiments were 125, 250 and 500 µg/ml. The amounts of gatifloxacin impurities used in these experiments were chosen based upon the results of cytotoxicity studies conducted under the same culture conditions as the chromosome aberration tests. The highest doses used were estimated to be slightly greater than the IC50 for gatifloxacin under the respective culture conditions, or were close to solubility limits in the culture medium in the case of the S-9 studies. Colcemid was added to all cell cultures 2 hours prior to the end of incubation. Cells were swollen in a hypotonic KCl solution, fixed with 3:1 ethanol/acetic acid, dropped onto slides, and stained with Giemsa. Each drug or control treatment was performed in duplicate and had its own slide. One hundred metaphase spreads per slide were examined, if possible. The positive control in the presence of S-9 was benzo[a]pyrene (BP, 10 µg/ml). N-methyl-N'-nitro-N-nitroso-guanidine (MNNG, 1 µg/ml) was the positive control in the absence of metabolic activation. The vehicle for the gatifloxacin impurities was saline (drug dissolved in 0.1 N NaOH, then neutralized with 0.1 N HCl) and the vehicle for the positive controls was DMSO. The negative control was the vehicle for the gatifloxacin impurities. The S-9 mix was derived from livers of male Crj:SD rats which had been treated with phenobarbital and 5,6-benzoflavone.

Results: Excluding gaps, the percentage of vehicle control cells with chromosome aberrations was 0.5-1.5% in each of the tests, regardless of incubation time or S-9. Treatment of CHL-IU cells with either the N-methyl (NM) or 2-methylpiperazine (2MP) gatifloxacin impurity caused a dose-dependant increase in chromosomal aberrations (including or excluding gaps) after 24 hours of in the absence of metabolic activation. Cells treated for 24 hours with NM at 90, 180, or 360 µg/ml had 5, 50, and 99% of cells with chromosome aberrations (excluding gaps) and those treated with 2MP for this time period at 88, 175, or 350 µg/ml had 0.5, 5, and 62.5% of cells with chromosome aberrations (excluding gaps). Results with 48 hour incubation were similar for NM (though the concentrations used were slightly lower, as were the percentages of cells at the low and mid dose with chromosome aberrations), but the 2MP form appeared not to have been active during the 48 hour incubation- perhaps the compound was not stable at 37°C for this period of time. The percentage of cells with chromosome aberrations excluding gaps was 1-2% at all concentrations of 2MP tested for 48 hours.

Results were more modest for the 6 hour incubation. For NM, the percentage of cells with chromosome aberrations (excluding gaps) were 2.5, 11.5, and 25% for 125, 250, and 500 µg/ml in the absence of S-9, and 1.5, 2, and 9.5% for the same concentrations in the presence of S-9. For 2MP (also at the same concentrations), the percentages were 1.5, 3, and 15.5% without S-9, and 0.5, 1, and 9% with S-9.

Positive controls behaved as expected for all tests.

The gatifloxacin impurities are clastogenic in Chinese hamster lung cells regardless of metabolic activation, but their clastogenic potential was decreased by co-incubation with S-9.

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

B6C3F1 mice given gatifloxacin in the diet for 18 months ~~at doses~~ ***with an average intake of*** up to 81 mg/kg/day in males and 90 mg/kg/day in females showed no increases in neoplasms.

2 pages of revised draft labeling have been redacted from this portion of the document.

[redacted]

SUMMARY AND EVALUATION: (much of this discussion has been taken directly from the pharmacologist's review of [redacted] under which many of the studies were reviewed)

Gatifloxacin is rapidly absorbed from the GI tract of mice, rats, rabbits, beagle dogs, and monkeys following oral administration. The bioavailability of gatifloxacin is highest in the beagle dogs and monkeys (about 90-100%), but lower in rats (55-93%), mice (52-63%), and rabbits (37-58%). Serum protein binding was low in all species (8-27%, with most values midway between the 2 extremes). The half life of gatifloxacin in mice and rats is 1-2 hr, in monkeys is about 2-2.5 hr, and in dogs is about 6 hours. Studies in rats using radiolabeled gatifloxacin demonstrated extensive tissue distribution following either IV or oral dosing with the exception that brain and CSF levels of gatifloxacin were very low. In contrast, gatifloxacin levels in brain tissue from mongrel dogs were about 50-70% of the serum level following an IV infusion where serum levels were held at around 3 μg/ml for 4 hours. A significant portion of gatifloxacin is excreted unchanged in the urine of many animals species such as mice (20%), rats (40-50%), rabbits (25-40%), dogs (40-45%), and monkeys (50-60%) after oral dosing. Studies in rats, also using oral administration, showed that some conjugated gatifloxacin is excreted in the urine (2-3% of the total dose) and that 14-17% of the total dose is excreted in bile (3-4% of total dose unconjugated with the rest in a conjugated form). The conjugated gatifloxacin in rats, rabbits and dogs was shown to be a glucuronide and three other metabolites (an ethylenediamine, a 2-methylethyenediamine, and an amino form of gatifloxacin) were also identified in the urine and feces of these species, although they each account for only about 1% of urinary or fecal gatifloxacin. Like many quinolones, gatifloxacin has a high affinity for melanin. When the drug was administered to pigmented rabbits and rats, it could be detected in melanin-containing ocular tissues at least 8 and 48 weeks following administration, respectively, though the levels were falling over time. Gatifloxacin did not appear to have a significant effect on theophylline metabolism in rats. The R- and S-enantiomers of gatifloxacin did not interconvert *in vivo* in rats, dogs, or monkeys. Both enantiomers and the racemate had similar pharmacokinetic parameters in mice, rats, rabbits, dogs, or monkeys.

Mortality did not occur after male and female Wistar rats were given single oral doses of gatifloxacin up to 2000 mg/kg and followed for 14 days. Clinical signs were observed in only one female animal and consisted of reduced activity, lowered breathing rate, and piloerection. All of these had resolved within 3 days after administration of drug. Administration of single 160, 400 or 1000 mg/kg doses of gatifloxacin to dogs was associated with salivation, vomiting, tremor, tonic convulsions, reduced spontaneous activity. The onset of vomiting was dose related, as was the severity and duration of the tremors and tonic convulsions. Lower skin temperature and ptosis were also observed at 1000 mg/kg. No mortality was observed in this acute dog study.

Mortality in rats was observed following single IV doses of gatifloxacin ≥144 mg/kg. Death occurred within 3 minutes of dosing and was preceded by tonic-clonic convulsions. Some survivors of the doses ≥144 mg/kg experienced symptoms of toxicity such as altered rate of respiration (either increased or decreased), reduction in spontaneous motor activity, salivation

tremor, and clonic convulsion. These symptoms were resolved by the morning following dosing, and in most cases, within 30 minutes to 2 hours of dosing. In male dogs, single IV doses of gatifloxacin up to 45 mg/kg did not cause mortality. No abnormal symptoms were observed following an IV 7.5 mg/kg dose, but higher doses were associated with suppression of spontaneous motor activity, tremor, salivation, collapse shortly after dosing and convulsion associated with increased respiration and decreased response to stimuli.

Rats dosed orally with 90 mg/kg gatifloxacin for one month demonstrated no drug-related adverse effects. The cecal enlargement observed at this dose (as well as the higher doses) is a nonspecific antibiotic effect. The deaths of one male each in the 270 and 810 mg/kg groups were likely to have been related to gatifloxacin treatment and appeared to have been due to intraperitoneal and gastrointestinal bleeding. Electron microscopy revealed liver changes including increased fat droplets, hepatocyte hypertrophy with irregular mitochondria, and an increase in Kupffer cells at both 270 and 810 mg/kg, but some of these effects may also be related to the nutritional status of the rats (a number of animals in the high dose group had reduced adipose tissue and reduced body weight gain was also observed at 810 mg/kg). Gatifloxacin-induced bone fragility, expansion of the epiphyseal plate, irregular arrangement of chondrocytes, and broken incisors were confined to the 810 mg/kg dose group. Vacuolation of pancreatic β-cells with dilatation of rough endoplasmic reticulum was also observed only in high dose rats; no changes in serum glucose were seen in these animals. The recovery group of 810 mg/kg rats provided evidence that the changes in liver, bone, cartilage, and pancreas appeared to be at least partially reversible within a 4 week drug-free period. In a 6 month oral study in rats, the lowest dose, 30 mg/kg, was associated with decreased testicular and epididymal weights and proliferation of smooth endoplasmic reticulum in hepatocytes in males. Proliferation of smooth ER in hepatocytes of female rats was not seen until doses of 120 and 240 mg/kg. Increases in hepatocellular lipid droplets were seen in rats from both genders at doses of gatifloxacin $\geq$60 mg/kg, but the change was more extensive in males. The pancreatic changes described in the one month rat study were observed in the 6 month study at doses $\geq$120 mg/kg. Although serum insulin levels in these rats was reported to be less than half of the control value, blood glucose levels did not differ from controls. Increased lipid mobilization may be occurring in these gatifloxacin-treated rats (note increase in hepatocellular lipid droplets mentioned above, as well as decreases in serum free fatty acids and triglycerides in females from the 2 highest dose groups). An increased incidence of fractured incisors was observed in males at 120 and 240 mg/kg in the 6 month study.

Significant mortality was observed in a one month IV gatifloxacin rat study at the high dose, 90 mg/kg. It was reduced to 60 mg/kg on day 17 of dosing, and no further mortality occurred. At necropsy, cecal enlargement was observed in the high dose rats, but no drug-related histopathological changes in liver, kidney or pancreas were noted. Other than local irritation at the site of injection which was observed in all drug-treated rats with a dose-dependent severity (dosing solutions of gatifloxacin ranged from 0.05-0.3%), clinical signs of toxicity were not observed in the 10 or 30 mg/kg dose groups.

A series of experiments in young adult rats were conducted to explore the bone fragility observed in these animals during the one month study. Over a 2 week dosing period, both bone and serum alkaline phosphatase activity, especially the former, were consistently lower in rats dosed orally with 810 mg/kg of gatifloxacin. After an initial reduction, urinary calcium excretion rose significantly (over 2 times control) over 2 weeks of dosing. The density (measured by

scanning an X-ray with a laser densitometer) of both compact and spongy bone fell 20-30% over the treatment period. Reductions in bone strength (40-70%, measured mechanically in femurs with a device referred to as an "autograph") and bone weight (12-16%) were apparent after one week of dosing. Bone length and bone volume were about 10% less than controls following 2 weeks of gatifloxacin treatment. After a 2 week recovery period, serum alkaline phosphatase levels were still higher than controls (by about 60%), but urinary calcium excretion was back to control levels. Bone length and bone weight were still 10-15% lower than control after recovery, but both bone density and bone strength were returning to control levels. In contrast, rats dosed orally with up to 90 mg/kg of gatifloxacin for 1 month did not demonstrate changes in bone parameters consistent with increased bone fragility, nor were there increases in urinary calcium excretion.

One month of daily oral gatifloxacin dosing at 7 mg/kg was associated with no drug-related adverse effects in beagle dogs. The poor condition of several animals in the 60 mg/kg group necessitated lowering the high dose to 40 mg/kg on day 13 of administration. Despite the lowered dose, 2 females died prior to the end of dosing and a third was sacrificed in moribund condition. Gatifloxacin-related clinical signs such as salivation and vomiting were observed at 20 and 60/40 mg/kg, and tremor, abnormal gait, and tonic convulsions were seen at 60/40 mg/kg. Gross lesions of the cartilage (dogs were 6-7 months old at study initiation and still considered juveniles) were observed in one animal at 20 mg/kg and in all high dose animals and microscopic examination of epiphyseal cartilage revealed irregular arrangement of chondrocytes in all animals from the mid and high dose groups. Necrosis of this tissue was also observed in most of these dogs. Vacuolation of pancreatic β-cells with dilatation of rough endoplasmic reticulum was observed in several mid and high dose animals and acinar atrophy was apparent in some high dose dogs. Light microscopy of the liver tissue showed only an increase in fatty droplets in one high dose female and hydropic degeneration in a second. Electron microscopy revealed liver changes in dogs from the 60/40 mg/kg group including increased fatty droplets in hepatocytes with irregular mitochondria, decreased glycogen granules and increased lysosomes. These effects, along with fatty droplets and swollen mitochondria in renal epithelium and atrophy of thymus and some male reproductive tissues may be related to the poor nutritional status of the high dose dogs, but lipid mobilization secondary to decreased insulin and reduced blood glucose utilization is also a possibility. Some dogs in the high dose group had reduced adipose tissue and reduced food consumption and body weight gain was observed at 60/40 mg/kg. The recovery group of 60/40 mg/kg dogs provided evidence that most histopathologic changes described above appeared to be at least partially reversible within a 4 week drug-free period. In the cartilage, gross lesions did not appear reversible, but the irregular arrangement of chondrocytes observed microscopically in the mid and high dose dogs did appear to be at least partially reversible. A 6 month oral study was also conducted in beagles. Pancreatic changes such as those described in the one month oral study were observed at 12 and 24 mg/kg, but not at 6 mg/kg. Decreased serum cholesterol and phospholipids were also observed in these dogs. As in the other studies, these could be due to reduced food consumption and body weight, or they could be secondary to reduced insulin and decreased blood glucose utilization with increased lipid mobilization. Cartilage blisters were seen in 2 dogs from the 6 and 24 mg/kg dose groups, but also in one control dog. These animals were one year old at the initiation of the study and would no longer be considered juveniles, so gatifloxacin-induced cartilage lesions would be less likely to occur in these dogs.

In a one month IV gatifloxacin study conducted in beagles, there did not appear to be any clinical signs of toxicity in the 7 mg/kg group and this dose was considered the NOEL. Vomiting and salivation were seen at the 2 higher dose groups, 15 and 30 mg/kg. Convulsions were seen on 1 or 2 occasions in some high dose dogs. Facial edema and decreased spontaneous movement were also seen at 30 mg/kg. Several female dogs dosed with 30 mg/kg of gatifloxacin had significantly fewer WBCs than control, with a reduction in the percentage of segmented neutrophils in particular. BUN was slightly elevated in several dogs and serum cholesterol and phospholipids were slightly to moderately reduced. Urinary excretion of potassium and chlorine was reduced in 4/12 animals of the 30 mg/kg group. Gatifloxacin-associated microscopic changes in liver, kidney, or pancreas were not observed.

Like other quinolones, gatifloxacin is associated with histamine release in dogs following an IV bolus dose of the drug. Gatifloxacin appeared to be less potent than ciprofloxacin in causing histamine release in the dog in a comparative study of the 2 drugs.

Mongrel dogs anesthetized with pentobarbital were used to determine the effect of gatifloxacin on respiration (rate, flow, volume) and cardiac parameters such as blood pressure (BP), heart rate (HR), EKG, and blood flow (BF, right femoral artery). A bolus IV injection of 1 or 3 mg/kg had no effect on respiration, BP, HR, or EKG. Femoral blood flow increased 40-150% immediately after dosing, but returned to normal within 20-40 minutes. After a bolus IV injection of 10 mg/kg, BP and HR decreased by about 50% and 20%, respectively, and had not returned 40 minutes following the dose. Within 3 minutes of injection, respiratory flow and volume decreased 20-30% and respiratory rate increased by 170%. Respiration was normal 30-40 minutes after dosing. Femoral blood flow doubled after dosing, fell rapidly, but then slowly increased once more. The R-R interval was prolonged and the QRS and T waves had slightly lowered amplitudes after bolus injection of 10 mg/kg. The same doses (1, 3, and 10 mg/kg) were also administered as continuous IV infusions of 30 minute duration. The 1 mg/kg dose did not appear to affect any of the respiratory or cardiac parameters measured. The 3 and 10 mg/kg doses did not seem to affect BP, HR, respiration or EKG. Femoral blood flow began to increase when the 3 and 10 mg/kg infusions began and were up to levels 40-50% greater than normal at the end of the infusion. The flow returned to normal within 30 minutes after the infusion was over.

In a 5 month oral gatifloxacin study conducted in cynomolgus monkeys, electron microscopy revealed vesiculation of rough endoplasmic reticulum and decreased secretory granules in pancreatic β cells from animals at all dose levels (15, 30, and 60 mg/kg). These effects were determined to be reversible, as they were not observed in monkeys which had received 60 mg/kg of gatifloxacin for 5 months, but allowed to recover for a 4 week drug free period prior to sacrifice. No treatment-related changes were observed in kidney or liver tissue from any monkey after 5 months of gatifloxacin administration. Soft stool was in the 2 higher dose groups. Dose-related reduced food consumption was also seen at 30 and 60 mg/kg, but reduced body weight gain was observed only at 60 mg/kg. One male in the high dose group showed erosion, hemorrhage and fibrosis of the right shoulder joint, but this may not be attributable to gatifloxacin as no other joint from this animal was abnormal, nor were the joints of any other monkeys in the study. These monkeys were adults and would not be expected to be as susceptible to quinolone-induced joint lesions as juveniles.

In a study conducted specifically to explore the cartilage toxicity induced by gatifloxacin in 4 month old beagle dogs, the NOEL for microscopic cartilage changes was 5 mg/kg when the

drug was given orally once per day for 7 days. Gross joint lesions (blisters) were observed only at the highest dose used (20 mg/kg), but microscopic examination revealed fibrous precipitation and degeneration of the epiphyseal cartilage matrix was seen in all dogs of both the 10 and 20 mg/kg groups. Other changes in the epiphyseal cartilage of some of the dogs in the 10 and 20 mg/kg dose groups included a decrease in proliferating cartilage cells, expansion of the epiphyseal plate, and irregular arrangement of hypertrophic cartilage columns.

A rabbit ear vein model was used to explore the venous irritation potential of gatifloxacin dosing solutions. The vehicle was physiologic saline. The test solutions were injected into an occluded auricular vein of New Zealand White rabbits daily for 8 days and occluded for 3 minutes for each injection. Dosing solutions up to 0.5% gatifloxacin were not irritating to the vein under the conditions of this study.

Gatifloxacin did not appear to affect the fertility of male or female rats at oral doses up to 200 mg/kg. No difference in mean testicular weights between the drug treated males and controls was seen. When administered orally to pregnant rats during fetal organogenesis, gatifloxacin caused no clinical signs in dams except for slightly reduced food consumption (but not body weight) at the high dose, 150 mg/kg. Fetuses and placentas from these dams weighed less than those in the control group and the incidences of wavy ribs and retarded ossification of the occipital bone and sternebrae were increased. A decrease in the number of ossification sites was also observed in the fetuses from the 150 mg/kg dose group. These signs indicate that 150 mg/kg of gatifloxacin given orally to pregnant rats during organogenesis is slightly fetotoxic; however, no drug-related visceral or skeletal malformations were observed in this pivotal study. A teratology study using the IV route of administration was also conducted in rats. Gatifloxacin caused no clinical signs in pregnant rats at IV doses up to 30 mg/kg/day given during organogenesis. In the F_1 fetuses, the incidences of wavy ribs and retarded ossification of sternebrae were increased at 30 mg/kg. These may indicate a slight fetotoxicity for gatifloxacin; however, no drug-related visceral or skeletal malformations were observed in the offspring in this pivotal study. In contrast, however, skeletal malformations were observed in pilot studies (not submitted to the agency until the NDA was filed) that were used to set the doses for the pivotal rat teratology studies. In the oral pilot rat teratology study, bent radius/ulna was seen in a greater percentage of drug-treated fetuses than controls. The percentage of fetuses (per total, not per litter) with this malformation was 0% in the control group and 4.7%, 8%, and 24.2% in the 200, 300, and 400 mg/kg dose groups, respectively. A bent scapula was seen in one fetus at 400 mg/kg. In the intravenous pilot rat teratology study, bent scapula was seen in two fetuses at 60 mg/kg (2.2% of total fetuses). Deformed humerus was seen in one fetus from the 60 mg/kg group (1.1% of total fetuses). These skeletal malformations were not observed in any control fetuses or in any other gatifloxacin-exposed fetuses (15 or 30 mg/kg). Based upon the data from these pilot studies, the investigators concluded that the highest appropriate dose to be used in the pivotal oral and intravenous rat teratology studies should be 150 and 30 mg/kg, respectively. The dams in the pivotal intravenous study had no clinical signs of toxicity at the 30 mg/kg dose level and neither food consumption nor body weight gain was less than control. Those in the pivotal oral study had only slightly reduced food consumption at 150 mg/kg, but no decrease in body weight gain compared to control was observed. As indicated above, signs of fetotoxicity (wavy ribs and retarded ossification of sternebrae) were observed in the high dose groups in the oral and intravenous rat teratology studies, but no visceral or skeletal malformation were seen (reviews of these studies are in [redacted] The reviewer disagrees that the highest appropriate dose

for the pivotal studies should have been 150 and 30 mg/kg. The dose-setting studies contained no data suggesting that 200 mg/kg (oral) or 60 mg/kg (IV) were inappropriately high doses for the dams or the fetuses. The reviewer cannot help but wonder whether the investigators chose lower doses due to the skeletal malformations observed at the higher doses. This type of study is supposed to be performed to explore the potential for a compound to induce fetal malformations. The highest doses used should be set at the maximum tolerated dose for the dams unless a lower dose is necessary to obtain a sufficient number of fetuses for examination (e.g., very high pre- or post-implantation loss occurs at doses well tolerated by the dam). A dose should not be chosen based upon having observed fetal malformations in the dose setting study that one would prefer not to see during a pivotal teratology study. Based upon the data from the oral and intravenous rat dose-setting studies, the reviewer believes that the skeletal malformations observed are probably drug-induced. The finding belongs in the label despite the negative results obtained in the pivotal studies where the high doses seem to have been set to avoid fetal malformations rather than unacceptably high toxicity to the dams. Additionally, the associate director for pharmacology and toxicology, Dr. Joseph DeGeorge, has advised the reviewer that the sponsor should be asked to repeat the rat teratology studies in question as a Phase 4 commitment, as it is not desirable to allow the labels to be written based upon data from nonGLP pilot studies when pivotal studies were deemed inadequate.

When gatifloxacin was administered orally to pregnant rabbits at doses up to 50 mg/kg during fetal organogenesis, the drug did not cause fetal abnormalities (variations or malformations). In a rat study of drug-induced peri- and post-natal effects (drug given daily from day 17 of pregnancy until day 21 of lactation), gatifloxacin caused no clinical signs in dams except for modestly reduced food consumption (but not body weight) at the high dose, 200 mg/kg. Newborn offspring from these dams weighed less than those in the control group and the incidences of wavy ribs and retarded ossification of the phalanges and vertebrae were increased in the pups that died prior to postnatal day 4. Postimplantation loss (late) was higher than control in dams from the 200 mg/kg gatifloxacin dose group and neonatal and perinatal mortalities for this high dose group were also greater than control. Despite continued gatifloxacin dosing of the dams, body weight gain in the surviving pups from the 200 mg/kg group was comparable to control and there was no delay in the acquisition of developmental landmarks in the high dose pups. The 200 mg/kg pups that were sacrificed at the end of the lactation period did not demonstrate drug-related visceral or skeletal abnormalities. There was no gatifloxacin-related dysfunction in the reproductive capacities of the F_1 rats in any dose group, and no abnormalities related to the drug were observed in their offspring (the F_2 pups). Gatifloxacin at maternal doses up to 200 mg/kg did not appear to cause behavioral abnormalities or learning deficits in the F_1 offspring. In this study, 200 mg/kg of gatifloxacin given orally to pregnant rats during the late stages of pregnancy and throughout lactation was toxic to the F_1 fetuses. The NOAEL in this study for F_1 fetotoxicity was 60 mg/kg. Pharmacokinetic studies in pregnant and lactating rats indicated that gatifloxacin crosses the placenta and is found in milk.

Gatifloxacin was associated with an increased mutation frequency at the HGPRT locus of V-79 cells and was clastogenic in Chinese hamster lung cells regardless of metabolic activation. It induced chromosome aberrations in human lymphocytes *in vitro*. Gatifloxacin did not induce reversion of the *Salmonella* TA98, TA100, TA1535, TA1537 or *E. coli* WP2*uvr*A-, but it should be noted that the concentrations used in this study were very low (< 0.5 µg/plate). In *S. typimurium* stain TA102, however, gatifloxacin was associated with an increase in the number of

revertants in the absence of metabolic activation (no study with S-9 was done with this strain) at concentrations of 8.8 - 44.4 ng/plate. This is consistent with data from other fluoroquinolones which also increased reversion in TA102, but not other bacterial strains. Gatifloxacin caused unscheduled DNA synthesis (UDS) in cultures of primary rat hepatocytes, but not cultured human lymphocytes. Unscheduled DNA synthesis was not induced in rat hepatocytes after the animals were given oral doses of gatifloxacin up to 2000 mg/kg. After 2 oral doses of gatifloxacin up to 500 mg/kg, mice showed no increase in the frequency of bone marrow polychromatic erythrocytes (PCEs) with micronuclei 24 hours after the second dose. After 2 IV doses of gatifloxacin up to 60 mg/kg, mice showed no increase in the frequency of bone marrow PCEs with micronuclei 24 hours after the second dose. Gatifloxacin did not induce chromosome aberrations in rat bone marrow cells *in vivo* after 2 consecutive daily doses up to 1000 mg/kg. It should be noted, however, that in the opinion of the pharmacology reviewer, the investigators could have successfully used a 2000 mg/kg dose in this study.

A carcinogenicity study was conducted in F344 rats fed a dietary admixture containing 0.06, 0.12 or 0.24% gatifloxacin for 104 weeks. Adrenal fatty change and pancreatic acinar atrophy with pancreatic duct proliferation were seen in the high dose males only. An increased incidence in LGL leukemia compared to control was observed in high dose males. However, this observation may not be biologically relevant to humans as LGL leukemia is a very common tumor in aging F344 rats. In a second carcinogenicity conducted in $B6C3F_1$ mice fed a dietary admixture containing 0.015, 0.03, or 0.06% gatifloxacin for 78-79 weeks, no gatifloxacin-related increases in benign or malignant tumors was observed.

Rats or guinea pigs receiving serum from animals sensitized to gatifloxacin did not demonstrate passive cutaneous anaphylaxis reactions when injected with either gatifloxacin alone or mixed with serum albumin. Serum from guinea pigs sensitized to gatifloxacin did not induce agglutination of sheep red blood cells coated with gatifloxacin and human serum albumin. In a test of active systemic anaphylaxis, none of the guinea pigs sensitized to gatifloxacin (via a series of IM injections) exhibited symptoms of anaphylaxis when challenged by IV injection with this drug, either by itself or mixed with human serum albumin. Photosensitization was not induced by gatifloxacin and UVA light in guinea pigs even when cyclophosphamide was used to increase the sensitivity of the method.

Mice and guinea pigs given single oral doses of gatifloxacin up to 500 mg/kg and mice given single intravenous doses up to 60 mg/kg and exposed to UVA radiation did not exhibit signs of phototoxicity. Gatifloxacin did not induce phototoxicity in the presence of UVA radiation in these studies, but the compound has its peak absorption in the UVB portion of the solar spectrum. Thus, the sponsor was advised to repeat phototoxicity testing using a light source that includes UVB (e.g., simulated sunlight). A phototoxicity study using simulated sunlight and male hairless mice demonstrated no evidence of phototoxicity when animals were given up to 800 mg/kg of gatifloxacin for two 5 day periods and exposed once (after the final dose) or twice (after dosing on day 5 and after the final dose) to UV.

The sponsor studied 2 gatifloxacin impurities (N-methyl gatifloxacin and 2-methylpiperazine gatifloxacin) for their acute and subchronic toxicities in rats after oral administration. Additionally, their genotoxic potential was studied using the Ames assay and a cytogenetics test in Chinese hamster lung cells. In the N-methyl group, single doses $\geq$250 mg/kg induced significant mortality in males and single doses $\geq$500 mg/kg induced significant mortality in females. Clinical signs observed in the rats included salivation, nasal hemorrhage, decreased

spontaneous activity, tremor, and convulsions. When 2-methylpiperazine was administered at doses up to 1000 mg/kg, there was no mortality. However, clinical signs in males at 500 and 1000 mg/kg included tremor, convulsions, and salivation. No clinical signs were observed in any of the females. The N-methyl and 2-methylpiperazine forms of gatifloxacin appear more toxic than the parent compound as the acute lethal dose in the rat for the parent was more than 2000 mg/kg for both genders and convulsions were not observed at this dose level. Lower doses of the impurities given for a longer period of time did not appear to increase the toxicity of gatifloxacin in the rat. Addition of N-methyl and 2-methylpiperazine impurities to gatifloxacin at levels of each did not increase the toxicity of gatifloxacin to rats at total daily oral doses of up to 270 mg/kg for 14-16 days. Neither gatifloxacin impurity induced reversion of the *Salmonella* or *E. coli* strains tested (TA98, TA100, TA1535, TA1537 and WP2*uvr*A-). However, due to the cytotoxicity of the gatifloxacin impurities (which appear to have antimicrobial activity), the concentrations used in this study were very low (generally less than 0.5 μg/plate). Similar to the parent compound, the gatifloxacin impurities were clastogenic in Chinese hamster lung cells regardless of metabolic activation, but their clastogenic potential was decreased by co-incubation with S-9.

Several of the toxic effects observed in animals following gatifloxacin administration are similar to those which have been seen with other quinolones. These include induction of arthropathy in juvenile dogs, convulsions and other CNS disturbances, and skeletal malformations and fetotoxicity in the offspring of female rats dosed with the drug. The observations of bone fragility in rats and vesiculation of rough endoplasmic reticulum and decreased secretory granules in pancreatic β cells of several species are less typical of the quinolones, though gatifloxacin may not be the only drug in this class that causes these effects. In particular, the specific pancreatic changes required the use of an electron microscope to be clearly identified in some of the studies and this technology is not used on a routine basis in studies conducted in the U.S. and Europe, in contrast to the Japanese studies presented here.

RECOMMENDATIONS: The pharmacologist does not object to the approval of this NDA. The nonclinical data for gatifloxacin are comparable to other quinolones marketed for clinical use. The label contains appropriate cautions regarding potential quinolone-related toxicities such as CNS effects, tendon rupture, and juvenile arthropathy. The sponsor should be requested to repeat the intravenous and oral rat teratology studies with adequate high dose levels as a Phase 4 commitment. The review team may wish to discuss other possible Phase 4 commitments that could be requested from the sponsor to explore the potential for QT prolongation by gatifloxacin in light of information that has been collected recently for other quinolones. At the request of the Executive Carcinogenicity Assessment Committee, the sponsor should be told that, regarding the mouse carcinogenicity study, an 18 month study with high survival is not generally acceptable for determining the carcinogenic potential of a pharmaceutical. However, the mouse study will be considered supportive evidence of the lack of gatifloxacin-induced carcinogenicity because the short term clinical use of this drug would not generally necessitate a carcinogenicity study and data from an adequate rat study with this compound are also available.

/S/

Amy L. Ellis, Ph.D.

Pharmacologist, HFD-520

Orig. NDA
cc:
HFD-520
HFD-590
HFD-104
HFD-340
HFD-520/Pharm Team Ldr/Osterberg
HFD-590/Pharm Team Ldr/Hastings
HFD-520/Pharm/Ellis
HFD-590/MO/Korvick
HFD-590/CSO/Atkins

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

/S/ 11/5/99 11/12/99

APPEARS THIS WAY
ON ORIGINAL

APPEARS THIS WAY
ON ORIGINAL

Histopathology Inventory for NDA # 21,062

Study	A97SR03
Species	Rat
Adrenals	X*
Aorta	X
Bone Marrow smear	X
Bone (femur)	X
Brain	X*
Cecum	X*
Cervix	
Colon	X
Duodenum	X
Epididymis	X*
Esophagus	X
Eye	X
Fallopian tube	
Gall bladder	
Gross lesions	X
Harderian gland	X
Heart	X*
Ileum	X
Injection site	
Jejunum	X
Kidneys	X*
Lachrymal gland	
Larynx	
Liver	X*
Lungs	X*
Lymph nodes, cervical	
Lymph nodes mandibular	X
Lymph nodes, mesenteric	X
Mammary Gland	X
Nasal cavity	
Optic nerves	X
Ovaries	X*
Pancreas	X
Parathyroid	X
Peripheral nerve	
Pharynx	
Pituitary	X*
Prostate	X*
Rectum	X
Salivary gland	X*
Sciatic nerve	X
Seminal vesicles	X*
Skeletal muscle	X
Skin	X
Spinal cord	X
Spleen	X*
Sternum	X
Stomach	X
Testes	X*
Thymus	X*
Thyroid	X
Tongue	X
Trachea	X
Urinary bladder	X
Uterus	X*
Vagina	X
Zymbal gland	

* organ weight obtained

Review and Evaluation of Pharmacology and Toxicology Data
Division of Anti-Infective Drug Products, HFD-520
CONSULTATION FOR HFD-590

NDA #: 21,061-000 (Oral); 21,062-000 (IV)

Date CDER Received: 12/28/98
Reviewer: Amy L. Ellis, Ph.D.
Date Assigned: 1/5/99
Number of Volumes: 2
Date Review Started: 8/27/99
Date 1ST Draft Completed: 9/1/99

KEY WORDS: Tequin, gatifloxacin, fluoroquinolone, oral, carcinogenicity, toxicokinetics

Sponsor: Bristol-Myers Squibb Company
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Wallingford, CT 06492
Phone: (203) 677-6883; Fax: (203) 677-7630

Authorized Representative: Douglas Kriesel, Ph.D.
Director, Worldwide Regulatory Affairs

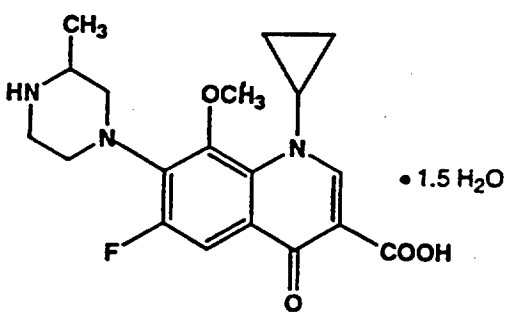
Manufacturer: Kyorin Pharmaceutical Company, Ltd.
Okaya Plant
Nagano, Japan

Review Contains Information to be Communicated to Sponsor: No

Submission Contains Any Integrated Tox Study Summaries in Lieu of Final Reports: No

Drug Information:

Class: Fluoroquinolone antimicrobial, DNA gyrase inhibitor
Code Names: BMS-206584; AM-1155; CG-5501
Generic Name: Gatifloxacin
Trade Name: Tequin
Chemical Name: 1-cyclopropyl-6-fluoro-1,4-dihydro-8-methoxy-7-(3-methyl-1-piperazinyl)-4-oxo-3-quinolinecarboxylic acid.
Structure:



Relevant INDs/NDAs/DMFs:

Indications: Community acquired pneumonia, acute exacerbation of chronic bronchitis, acute sinusitis, complicated and uncomplicated urinary tract infections, uncomplicated skin and skin structure infections, gonococcal infections. The daily recommended dose of gatifloxacin will be 400 mg/day for 7-14 days for most of these indications. The exceptions are uncomplicated UTI and gonococcal infections where the recommended doses will be 200 mg/day for 3 days or a single dose of 400 mg.

Clinical Formulation/Routes of Administration:

Each 200 mg Tequin Oral Tablet contains:

Gatifloxacin	200 mg
Microcrystalline Cellulose	
Sodium Starch Glycolate	
Magnesium Stearate	
Coating Suspension Solids*	
Total Tablet Weight	306 mg

*Composition of Coating Suspension (per 200 mg tablet):

Each 400 mg Oral Tablet contains twice as much of each ingredient as the 200 mg tablets contain.

Tequin I.V. will be packaged in single use vials containing 200 mg (20 ml) or 400 mg (40 ml) of gatifloxacin in a sterile, preservative-free solution. The contents of the vials are intended to be diluted to 2 mg/ml in an appropriate infusion solution prior to use. Each ml of this solution for intravenous use contains:

Gatifloxacin	10 mg
Dextrose (anhydrous)	
HCl and/or NaOH	
Water for Injection	

Tequin I.V. will also be available in a ready to use, preservative-free formulation of 2 mg/ml gatifloxacin in 5% dextrose. This solution will be packaged in flexible infusion bags in a 100 ml or 200 ml volume.

Introduction and Drug History: Gatifloxacin is a member of the fluoroquinolone antimicrobial group and is an inhibitor of bacterial DNA gyrase. Like some of the other newer quinolones, it can be administered once daily and has a broader spectrum of antimicrobial activity than many of the older drugs in this class (active against gram positive organisms as well as gram negative). The sponsor has submitted data suggesting that gatifloxacin has a much lower phototoxic potential than some of the other quinolones. Many of the nonclinical toxicity studies conducted with gatifloxacin have been performed by Kyorin Pharmaceutical Company in Japan. Bristol-Myers Squibb obtained the right to develop and market gatifloxacin from Kyorin.

This review will include the mouse carcinogenicity study conducted with gatifloxacin, the toxicokinetics report for this study, and the toxicokinetics report for a rat carcinogenicity study that was reviewed previously. The protocol for the mouse carcinogenicity study was not submitted to the division prior to its conduct.

Dietary Carcinogenicity Study in Mice (INA Study No. KS95012) (BMS Report No. 910065003)

Report dated: 5/20/97, Japanese [redacted] with translation of signed QA statement; Start of administration: 3/28/95; Date of final sacrifices: 10/4/96

Vols. 34 and 35

Animals: Slc:B6C3F$_1$ mice, approximately 8 weeks old at the initiation of dosing, 17.0-25.1 g (males) and 15.2-21.8 g (females), housed individually, 50/sex per dose group for the carcinogenicity portion of the study and 20/sex per group for determination of gatifloxacin blood levels, 9-12 extra animals/sex per group were used for periodic routine monitoring for microorganisms

Diet: CE-2 powdered food (with or without gatifloxacin, as applicable) and tap water were available *ad libitum*. Mice in the carcinogenicity portion of the study were fasted overnight prior to scheduled sacrifice.

Drug Doses and Route of Administration: Gatifloxacin (Lot. No. G3X5321) was administered as a dietary admixture. A control group received powdered food without drug. The concentrations of drug in the diet were 0.015, 0.03, and 0.06%. Analytical testing showed that the actual concentrations were within 93.0-103% of target and mixtures were uniform. The mixtures were shown to be stable for up to 3 months when protected from light. They were used within 12 weeks of preparation. The mean doses achieved in the animals were 19.8, 39.8, 81.4 mg/kg/day for the males and 22.4, 43.8, and 89.7 mg/kg/day for the females. These doses were chosen based upon the results of a 13-week dietary study where a 12% reduction in body weight gain was observed in males fed a diet containing 0.12% gatifloxacin. The highest proposed clinical dose of drug is 400 mg/day for up to 14 days. In a 60 kg adult, this would be about 6.7 mg/kg/day.

Conduct and Length of Study: Males were given drug for 78 weeks and females for 79 weeks. They were observed twice daily for clinical signs and palpated weekly to determine whether masses were present. Food consumption was measured weekly. Body weights were reported weekly for the first 14 weeks, then every 4 weeks for the rest of the study. Blood samples for hematologic evaluation were collected at the time of sacrifice and bone marrow smears were taken from the femur. All tissues (see histopathology table) collected from the mice in the control and high dose groups were examined microscopically. Only tissues with gross lesions were examined microscopically for the low and intermediate dose groups.

It should be noted that the sponsor did not consult with the Division prior to performing this study and the Division did not request that the carcinogenic potential of gatifloxacin be examined. The sponsor conducted the study according to a protocol of their own devising.

Results: One female in the control group was accidentally killed on day 15. This animal was not replaced and was excluded from the study.

Treatment with gatifloxacin did not alter the mean survival time compared to control. The mean survival periods of the control mice were 77.9 weeks for males and 78.5 weeks for females. The mean survival periods for the mice fed a diet containing gatifloxacin ranged from 76.5 to 77.4 for the males and 77.7 to 78.8 weeks for the females. Prior to death, clinical signs such as emaciation, gyration, decreased movement, piloerection, and irregular respiration were observed in some mice as their condition deteriorated. These signs did not appear to be specifically related to gatifloxacin treatment. Hair loss was observed at similar frequency in all groups.

Number of Mice (out of 50*) Surviving Until the End of the Study (78 Weeks- Males and 79 Weeks-Females)

Amount of Drug in Diet	Males	Females
0% (Control)	48	46
0.015%	48	48
0.03%	47	49
0.06%	45	49

*out of 49 for control females

Mean body weights of drug-treated male mice were not significantly different than control at the end of the study. Occasionally during the study, the mean body weight of the high dose males was statistically significantly lower than control, but the difference between the two groups was never more than 5%, so the reviewer does not believe that the differences were biologically significant. Mean body weights of female mice from the 0.015% and 0.06% dose groups were statistically significantly lower (about 8%) than control at the end of the study. Beginning at week 42, mean body weight of the 0.06% dose group tended to be about 7.5-9% less than control with statistically significant differences shown on several occasions. At the end of the study, mean body weight of the 0.03% female group was only about 3% less than control (no statistically significant difference). Drug-related differences in food consumption were not observed.

No biologically significant differences in hematologic parameters were observed in the gatifloxacin-treated mice compared to controls. Clinical chemistry testing and ophthalmoscopy were not part of this study protocol.

Gatifloxacin treatment was not associated with the development of palpable masses in the mice, nor was it associated with a general increase in the number of animals with either benign or malignant neoplasms. A table from the study report enumerating histopathologically confirmed neoplastic lesions observed in the mice is appended to this review. There did not appear to be any drug-related increases in any particular tumor type. There was a statistically significant decrease in the incidence of malignant lymphoma in the female mice from the 0.06% gatifloxacin group compared to control. Upon gross pathological examination, the incidence of uterine cysts was higher in the drug-treated rats (33, 34, and 36 mice in the low, medium, and high dose groups, respectively) than controls (24 mice). Microscopic examination of the uterine tissue, however, revealed a similar incidence and histologic severity of uterine cysts among all groups of female mice, including controls. No treatment-associated increases in the incidence of non-neoplastic histologic changes were observed in the mice.

Number of Mice (out of 50*) with Benign or Malignant Neoplastic Lesions (Confirmed Microscopically)

% Gatifloxacin in Diet	Number of Mice with Malignant Neoplasms	Number of Mice with Benign Neoplasms	Number of Mice with Any Neoplasm
Males			
0% (Control)	5	25	29
0.015%	0	30	30
0.03%	3	26	28
0.06%	10	30	32
Females			
0% (Control)	8	10	17
0.015%	2	13	15
0.03%	2	7	8
0.06%	3	6	9

*out of 49 for control females

There was a significant increase in the mean absolute and relative and cecal weights (about 13-25%) in all groups of gatifloxacin-treated mice regardless of gender compared to controls. This is a common finding in mice treated with antibiotics. No corresponding microscopic changes in the cecal tissue were reported by the pathologist. Significant increases in both absolute and relative uterine weights were observed in the 0.06% females (36-46%), perhaps secondary to the uterine cysts observed upon gross and microscopic examination. A statistically significant decrease in absolute and relative kidney weights (5-7%) was observed in the males from the 0.06% gatifloxacin dose group. However, no corresponding microscopic renal changes were reported.

Gatifloxacin was not carcinogenic in male or female B6C3F$_1$ mice when administered in the diet at concentrations up to 0.06%. It should be noted, however, that the maximum tolerated dose was not achieved in this study.

Toxicokinetics of Gatifloxacin During a 2-Year Carcinogenicity Study of Gatifloxacin in Mice, Study No. KS95012 (BMS Report No. 910062408)

H. Kusajima, S. Manita, M. Machida, R. Ishida (Kyorin Pharmaceutical Co., Ltd., Tokyo, Japan)

Report dated 10/11/96 and signed by investigator 10/16/96

Vol. 54, pp. 20-37

Summary: This is the toxicokinetics report for the mouse carcinogenicity study reviewed above. [redacted] was used to detect gatifloxacin in the serum of mice (3 animals per sex sampled at each time point). Blood samples were drawn in the morning and evening (9-10 AM and 5-6 PM) 1, 6, and 12 months after the initiation of dosing. Frozen serum was sent to Kyorin by the contract laboratory that conducted the carcinogenicity study [redacted]

Serum concentrations of gatifloxacin increased in a dose-dependant manner and there did not appear to be any gender-based differences among the groups. The serum concentrations were higher at 1 and 6 months than they were at 12 months.

Serum Concentrations of Gatifloxacin During a Mouse Carcinogenicity Study (μg/ml, average ± SD)

Month		0.015%		0.03%		0.06%	
		Male	Female	Male	Female	Male	Female
1	AM	0.10 ± 0.01	0.08 ± 0.01	0.17 ± 0.04	0.16 ± 0.00	0.28 ± 0.10	0.24 ± 0.03
	PM	0.06 ± 0.01	0.09 ± 0.02	0.12 ± 0.02	0.21 ± 0.01	0.29 ± 0.03	0.43 ± 0.16
6	AM	0.07 ± 0.02	0.12 ± 0.08	0.15 ± 0.00	0.19 ± 0.02	0.31 ± 0.07	0.39 ± 0.10
	PM	0.05 ± 0.01	0.08 ± 0.02	0.10 ± 0.02	0.20 ± 0.02	0.16 ± 0.03	0.33 ± 0.08
12	AM	0.06 ± 0.00	0.06 ± 0.01	0.11 ± 0.02	0.13 ± 0.02	0.20 ± 0.03	0.30 ± 0.14
	PM	0.05 ± 0.02	0.05 ± 0.01	0.07 ± 0.03	0.13 ± 0.06	0.15 ± 0.02	0.19 ± 0.03

Toxicokinetics of BMS-206584 (AM-1155) During a 2-Year Carcinogenicity Study of BMS-206584 in Rats, Study No. T-9305 (BMS Report No. 910058592)

H. Shiina, T. Tachiiri, K. Momo, H. Kusakawa, H. Kusajima, R. Ishida (Kyorin Pharmaceutical Co., Ltd., Tokyo, Japan)

Report dated 9/30/96 and signed by investigator 10/1/96

Vol. 54, pp. 64-77

Summary: This is the toxicokinetics report for the rat carcinogenicity study (C92GR20) reviewed by Dr. Terry Peters and filed under [redacted] The rats were fed a diet containing gatifloxacin at concentrations of 0.06, 0.12, or 0.24%. [redacted] was used to detect gatifloxacin in the serum of rats (3 animals per sex sampled at each time point during weeks 26 and 51; 2 animals per sex sampled during week 103). Blood samples were drawn at approximately 11 AM.

Serum concentrations of gatifloxacin increased with dose. There did not appear to be any gender-based differences within the two lower dose groups, but concentrations in the females were higher than males at the 0.24% dose level. The serum concentrations tended to be lower at 103 weeks than they were at the earlier time points.

Serum Concentrations of Gatifloxacin During a Rat Carcinogenicity Study (μg/ml, average ± SD)

Week	Gender	0.06%	0.12%	0.24%
26	M	0.24 ± 0.02	0.50 ± 0.01	0.97 ± 0.15
	F	0.29 ± 0.02	0.48 ± 0.10	1.53 ± 0.15
51	M	0.36 ± 0.21	0.43 ± 0.03	0.98 ± 0.10
	F	0.27 ± 0.01	0.56 ± 0.14	1.35 ± 0.07
103	M	0.22	0.37	0.67
	F	0.19	0.35*	0.59*

*only one rat at this time point

OVERALL SUMMARY AND EVALUATION:

Gatifloxacin was not carcinogenic to mice when administered in the diet for 78-79 weeks at concentrations up to 0.06%. The average dose of gatifloxacin delivered to the high dose mice over the course of the study was 80-90 mg/kg. The maximum tolerated dose was not achieved in this study. However, data from a 3 month dose setting study indicates that the highest dose used for the mouse carcinogenicity study was probably within a factor of 2 of the MTD. In the dose setting study, male mice had about a 9% reduction in body weight gain compared to controls when fed a diet containing 0.1% gatifloxacin, and mortality secondary to cecal torsion occurred in 3/10 male mice from this dose group. Mortality was not observed in the female mice in the first dose setting study until the concentration of gatifloxacin in the diet reached 0.2% and a decrease in body weight-gain was not observed until 0.4%. However, gross necropsy revealed cecal torsion in all of the surviving male mice and 7/10 female mice in the 0.1% gatifloxacin dose group. It is unlikely that these mice would have survived for an adequate length of time for a chronic study. Dose-related cecal torsion is a common finding in mice fed diets containing anti-infective compounds and is frequently a dose-limiting toxicity in chronic studies with this species.

In adult humans, the Cmax of gatifloxacin is approximately 4.2 μg/ml following multiple 400 mg oral doses and approximately 4.6 μg/ml following multiple 400 mg IV doses. Regardless of the route of administration, the AUC is about 35 μg·hr/ml after a 400 mg/kg dose is given. The highest plasma levels measured in mice and rats receiving gatifloxacin in the diet during

carcinogenicity studies were approximately 0.4 and 1.5 μg/ml, respectively. For comparative purposes the AUC in male ICR mice following a 10 mg/kg oral dose of gatifloxacin was about 2.7 μg·hr/ml. The highest average dose of gatifloxacin delivered in the $B6C3F_1$ mouse carcinogenicity study was about 80-90 mg/kg (0.06% drug in the diet). The plasma concentrations in the mice increased in a dose-dependant manner in the carcinogenicity study, so assuming that would also be true for AUC (and ignoring any metabolic differences between strains of mouse), one would estimate an average AUC of about 22-24 μg·hr/ml in the mice from the 0.06% dose group. In male Wistar rats, the AUC after a 10 mg/kg oral dose of gatifloxacin was about 8.5 μg·hr/ml. The highest average doses of gatifloxacin delivered in the F344 rat carcinogenicity study were about 100 and 140 mg/kg, in males and females, respectively. The plasma concentrations for the rats increased in a generally dose-dependant manner in the carcinogenicity study, so assuming that would also be true for AUC (and ignoring any metabolic differences between strains of rat), one would estimate an average AUC of about 85-120 μg·hr/ml. Thus, exposure to gatifloxacin in the high dose rodents was about 3-10 times less than in humans based upon Cmax. Based upon estimations of AUC, the high dose mice in the carcinogenicity study received about 60% of the daily human exposure and the high dose rats received about 2.4-3.4 times the daily human exposure. It should be noted that humans will receive gatifloxacin for up to 14 days per course of therapy, not chronically as the rodents did in the carcinogenicity studies.

RECOMMENDATIONS: The data from the carcinogenicity studies with gatifloxacin should be included in the product's label.

/S/

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

Concurrence Only:
HFD-520/REOsterberg 9/28/99
HFD-520/LGavrilovich /S/ 9/29/99

Orig. NDA
cc:
HFD-520
HFD-590
HFD-104
HFD-340
HFD-520/Pharm Team Ldr/Osterberg
HFD-590/Pharm Team Ldr/Hastings
HFD-520/Pharm/Ellis
HFD-590/MO/Korvick
HFD-590/CSO/Atkins

Histopathology Inventory

Study	KS9501
Species	$B6C3F_1$ mice
Adrenals	X*
Aorta	X
Bone Marrow smear	X
Bone (femur)	X
Brain	X*
Cecum	X*
Cervix	
Colon	X
Duodenum	X
Epididymis	X*
Esophagus	X
Eye	X
Fallopian tube	
Gall bladder	
Gross lesions	X
Harderian gland	X
Heart	X*
Ileum	X
Injection site	
Jejunum	X
Kidneys	X*
Lachrymal gland	X
Larynx	X
Liver	X*
Lungs	X*
Lymph nodes, cervical	
Lymph nodes mandibular	X
Lymph nodes, mesenteric	X
Mammary Gland	X
Nasal cavity	X
Optic nerves	X
Ovaries	X*
Pancreas	X
Parathyroid	X
Peripheral nerve	
Pharynx	
Pituitary	X*
Prostate	X*
Rectum	X
Salivary gland	X*
Sciatic nerve	X
Seminal vesicles	X*
Skeletal muscle	X
Skin	X
Spinal cord	X
Spleen	X*
Sternum	X
Stomach	X
Testes	X*
Thymus	X*
Thyroid	X
Tongue	X
Trachea	X
Urinary bladder	X
Uterus	X*
Vagina	X
Zymbal gland	

* organ weight obtained

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Table 17-1 Histopathological findings in male mice administered AM-1155 orally for 18 months
- Neoplastic lesion -

Organ and tissue ; Findings	Group	Control	AM-1155 (%) 0.015	0.03	0.06
	Number of animals	50 [2]	42 [2]	38 [3]	50 [5]
Hematopoietic organs ;		<50> [2]	[2]	[3]	<50> [5]
Malignant lymphoma (M)		1 [0]	[0]	[0]	2 [0]
Malignant mast cell tumor (M)		0 [0]	[0]	[0]	1 [0]
Lungs (Bronchi) ;		<50> [2]	< 9> [2]	<10> [3]	<50> [5]
Alveolar/bronchiolar carcinoma (M)		0 [0]	0 [0]	2 [0]	1 [1]
Alveolar/bronchiolar adenoma (B)		6 [0]	6 [0]	5 [1]	4 [0]
Spleen ;		<50> [2]	[2]	[3]	<49> [4]
Histiocytic sarcoma (M)		1 [0]	[0]	[0]	0 [0]
Hemangioma (B)		0 [0]	[0]	[0]	2 [0]
Liver ;		<50> [2]	<30> [2]	<27> [3]	<50> [5]
Hepatocellular carcinoma (M)		3 [1]	0 [0]	0 [0]	2 [0]
Hepatocellular adenoma (B)		22 [0]	26 [0]	20 [0]	22 [2]
Hemangioma (B)		0 [0]	0 [0]	2 [0]	0 [0]
Stomach ;		<49> [1]	[2]	< 4> [3]	<48> [3]
Squamous cell carcinoma (M)		0 [0]	[0]	1 [0]	0 [0]
Pituitary ;		<49> [1]	< 3> [2]	[1]	<50> [5]
Adenoma (B)		0 [0]	1 [0]	[0]	0 [0]
Thyroid ;		<50> [2]	[2]	[3]	<49> [4]
C-cell carcinoma (M)		0 [0]	[0]	[0]	1 [0]
Follicular cell adenoma (B)		1 [0]	[0]	[0]	0 [0]
Adrenal ;		<50> [2]	[2]	[3]	<49> [4]
Cortical adenoma (B)		0 [0]	[0]	[0]	1 [0]
Harderian gland ;		<50> [2]	[2]	[2]	<50> [5]
Adenoma (B)		0 [0]	[0]	[0]	3 [0]
Skin/Subcutaneous tissue ;		<50> [2]	<12> [2]	<13> [3]	<50> [5]
Squamous cell papilloma (B)		0 [0]	0 [0]	0 [0]	1 [0]
Hemangioma (B)		0 [0]	0 [0]	0 [0]	1 [0]
Preputial gland ;		<50> [2]	[2]	[3]	<50> [5]
Squamous cell carcinoma (M)		0 [0]	[0]	[0]	2 [1]
Subperitoneal mass ;		-	-	-	< 1> [0]
Hemangioma (B)		-	-	-	1 [0]
Abdominal wall ;		-	-	-	< 1> [0]
Lipoma (B)		-	-	-	1 [0]
Mesothelium ;		-	-	-	< 1> [0]
Mesothelioma (M)		-	-	-	1 [0]

[ ] : Number of dead animals
< > : Number of animals examined
- : Not examined
(M) : Malignant tumor (B) : Benign tumor

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Table 17-2 Histopathological findings in female mice administered AM-1155 orally for 18 months

- Neoplastic lesion -

Organ and tissue ; Findings	Group / Number of animals	Control	AM-1155 (%) 0.015	0.03	0.06
		50 [4]	43 [2]	40 [1]	50 [1]
Hematopoietic organs ;		<50> [4]	[2]	[1]	<49> [0]
Malignant lymphoma (M)		7 [2]	[0]	[1]	0 [0] **
Lungs (Bronchi) ;		<50> [4]	< 6> [2]	< 4> [1]	<50> [1]
Alveolar/bronchiolar carcinoma (M)		0 [0]	0 [0]	1 [0]	0 [0]
Alveolar/bronchiolar adenoma (B)		1 [0]	3 [0]	2 [0]	1 [0]
Kidney ;		<50> [4]	< 3> [2]	[1]	<50> [1]
Tubular cell carcinoma (M)		0 [0]	0 [0]	[0]	1 [0]
Tubular cell adenoma (B)		0 [0]	1 [0]	[0]	0 [0]
Spleen ;		<50> [4]	[2]	[1]	<49> [0]
Histiocytic sarcoma (M)		0 [0]	[0]	[0]	1 [0]
Liver ;		<50> [4]	< 8> [2]	< 4> [1]	<50> [1]
Hepatocellular adenoma (B)		7 [0]	6 [0]	3 [0]	2 [0]
Stomach ;		<48> [2]	< 3> [2]	[1]	<49> [0]
Squamous cell papilloma (B)		1 [0]	0 [0]	[0]	0 [0]
Ovary ;		<50> [4]	< 4> [1]	< 6> [1]	<49> [0]
Cystadenoma (B)		1 [0]	0 [0]	2 [0]	1 [0]
Uterus ;		<50> [4]	<37> [2]	<35> [1]	<49> [0]
Leiomyoma (B)		0 [0]	0 [0]	0 [0]	1 [0]
Pituitary ;		<49> [3]	< 3> [1]	< 2> [1]	<49> [0]
Adenocarcinoma (M)		0 [0]	0 [0]	0 [0]	1 [0]
Adenoma (B)		0 [0]	2 [0]	1 [0]	0 [0]
Thyroid ;		<49> [3]	< 3> [2]	< 2> [1]	<50> [1]
Follicular cell adenoma (B)		0 [0]	1 [0]	0 [0]	1 [0]
Skin/Subcutaneous tissue ;		<50> [4]	< 6> [2]	< 3> [1]	<50> [1]
Leiomyosarcoma (M)		1 [1]	0 [0]	0 [0]	0 [0]
Fibrosarcoma (M)		0 [0]	1 [0]	0 [0]	0 [0]
Femur ;		<50> [4]	[2]	[1]	<50> [1]
Osteosarcoma (M)		0 [0]	[1]	[0]	0 [0]

[ ] : Number of dead animals

< > : Number of animals examined

(M) : Malignant tumor (B) : Benign tumor

** Significantly different from control (P<0.01)