

NDA 21,158-000/Factive (gemifloxacin)

Increased water consumption was seen in males at 20 mg/kg and signs of a slightly increased plasma volume were also observed in the rats from this dose group (slight decreases in red cell parameters and plasma protein and globulin concentrations). The hematologic changes were no longer evident after the 4 week recovery period and their magnitude was too small for them to be of biological significance. In contrast to a previous 4 day IV study in rats (which used doses as high as 60 and 100 mg/kg/day) hypocellularity of bone marrow in the sternum (associated with decreased reticulocytes and white blood cells in the peripheral blood) and pericholangitis were not observed in the current 28 day study. Renal tubular nephropathy was observed in 10/10 males and 1/10 females from the 20 mg/kg group and 4/10 males in the 10 mg/kg group. Microscopic changes in the kidneys included basophilia of the tubular epithelium, tubular dilatation, interstitial inflammatory cell infiltration, and papillary epithelial hyperplasia. Plugs in the distal tubules and/or renal pelvis of rats from the 10 and 20 mg/kg dose groups appeared similar to those seen in the previous studies that were shown to contain drug-related material. Drug-related crystal nephropathy has been seen in rats when other quinolones were administered. The NOAEL for gemifloxacin administered IV daily for 28 days was 2 mg/kg.

SB 265805: Toxicokinetics Followed by a 14-Day Fixed Dose Oral (Capsule Administration) Toxicity Study in the Dog (SB Document No. SB-265805/RSD-1013RW/1; LG Chem Study No. PKL20304-08)

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Report dated 6/3/99

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Summary: This is the toxicokinetics report for the "fixed dose" portion of Study No. 1405/6-1050 conducted at _____ (reported in SB Document No. SB-265805/RSD-100LK5/2 and reviewed under IND _____). Beagle dogs received 500 mg/kg of gemifloxacin (Batch No. Q002) in gelatin capsules for days 1-6, they were not dosed on days 7-8, and they received 400 mg/kg on days 9-22 (the dose had to be lowered due to reduced food consumption and weight loss). Blood samples for toxicokinetics were taken on days 1 and 22 immediately prior to dosing, then 10, 20, and 40 minutes and 1, 1.5, 2, 3, 5, 8, 12, and 24 hours after administration of gemifloxacin. Serum concentrations were measured using _____.

Toxicokinetics of Gemifloxacin after a Single Dose of 500 mg/kg

	Male 1	Male 2	Female 1	Female 2
Tmax (min)	180	300	300	300
Cmax (g/ml)	6.05	7.10	6.51	8.01

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AUC _{0-24h} (ghr/ml)	86.1	92.9	83.4	92.7
T _{1/2} (h)	10.1	9.6	7.9	7.4

Toxicokinetics of Gemifloxacin after Multiple 400 mg/kg Doses

	Male 1	Male 2	Female 1	Female 2*
T _{max} (min)	180	180	180	40
C _{max} (g/ml)	5.06	9.43	6.59	7.92
AUC _{0-24h} (ghr/ml)	55.0	99.3	67.8	34.8
T _{1/2} (h)	9.2	5.5	5.5	6.9

*Dog vomited 45 minutes after dosing

SB 265805: 13-Week Oral (Capsule Administration) Toxicity Study in the Dog (SB Document No. SB-265805/RSD-100Z4L/1; Protocol No. G98612; Study No. 802/480)

Report dated 8/20/99, and GLP

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Animals: Beagle dogs 10-12 months old and 6.20-12.85 kg (males) or 6.20-9.80 kg (females) on the first day of dosing, housed in pairs by sex and treatment group, 4/sex per dose group

Diet: Dog Maintenance Diet (400 g/dog) was offered daily and tap water was available *ad libitum*.

Drug Dose and Route of Administration: Gemifloxacin mesylate (Batch No. EF03-12R1P5) was placed into gelatin capsules and administered orally once per day at dose levels of 0 (empty capsules), 5, 30, and 120 mg/kg/day (based on the salt). The 120 mg/kg dose was not administered on days 37 and 38 of the study and the group received 60 mg/kg/day beginning on day 39 for the duration of the study.

Length and Conduct of Study: Drug was administered daily for 13 weeks with dogs receiving the final dose on the day before sacrifice.

The dogs were checked for viability twice daily. Clinical signs were recorded each day before administration of drug, after the dose was given, and twice more during the day (3-7 hours after dosing). The animals were weighed once per week (except high dose dogs were weighed daily from days 37-42 and 45-52). Food consumption was monitored daily, but calculated as g/dog per week. examinations (direct and indirect) were performed prior to the initiation of dosing and during week 13. Blood

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samples (for the determination of hematologic and clinical chemistry parameters) were drawn before the period of drug treatment and during weeks 1, 4, 8, and 13. A week 6 blood sample was also drawn from the control and high dose dogs.

Blood for toxicokinetic evaluation was drawn from each dog on day 1 and during weeks 4 and 13 of the study 0.5, 1, 2, 4, 8, 12, and 24 hours after dosing. Plasma levels of individual gemifloxacin enantiomers were measured using _____ with a lower limit of quantification of _____ ng/ml for each enantiomer.

At necropsy, a 20 g liver sample was taken from each dog and frozen in _____ buffer (pH 7.4) for analysis of hepatic cytochrome P450. Results of that evaluation were reported separately.

In addition to the tissues recorded on the histopathology checklist, the elbow and stifle joints and a lumbar vertebra were harvested and grossly examined. Only the liver, knee, and elbow joints were examined microscopically, however.

Results: One female in the 120/60 mg/kg group was sacrificed in moribund condition during week 6. This dog had reduced food consumption and lost 1.2 kg of its body weight during the dosing period. Blood samples drawn 2 days before sacrifice showed elevated ALT, AST, SDH and direct bilirubin levels. The levels of these hepatic markers for this dog were even greater than those for the rest of the animals in the high dose group, which also had elevations in these parameters. Microscopic examination of the dog's liver tissue revealed severe vacuolation of hepatocytes, amorphous biliary deposits, and biliary proliferation.

Vomiting was observed occasionally in the 120/60 mg/kg group, usually 0.5-4 hours after dosing. Vomiting was seen at a lower incidence with less frequency at 30 mg/kg. Beginning during week 5, salivation was observed in the 120/60 mg/kg dogs immediately before dosing or shortly after.

Significant body weight loss occurred at 120mg/kg (250-1850 g in the males and 1200-1800 g in the females), so dosing was suspended on days 37 and 38. Food consumption was significantly reduced (by as much as half) during weeks 4 and 5, particularly the latter. Administration of gemifloxacin resumed on day 39 at 60 mg/kg. The dogs gained weight (200-1000 g) as soon as the 120 mg/kg dose was stopped and food consumption improved immediately, so that it was comparable to control for the rest of the study. During the period when these animals received 60 mg/kg, body weight gain was similar to control. The 5 and 30 mg/kg doses had no significant effect on body weight or food consumption compared to control.

Gemifloxacin did not appear to affect hematologic parameters. Serum clinical chemistry values which were indicative of hepatic function were affected by gemifloxacin treatment, particularly the 120/60 mg/kg males. During week 1 of treatment, two 120 mg/kg males had moderately increased (compared to control and baseline) levels of ALT, GGT, and ALP, with one of the dogs having elevated AST as well. Elevated ALT was also observed in two other dogs (one male, one female) from this dose group and in a male and a female from the 30 mg/kg dose group. The 30 mg/kg dogs also had slightly high SDH activities and the male had slightly high AST and ALP as well. By week 4, all 120 mg/kg males and 3/4 of the females had high ALT (males

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were all in excess of 300 IU/l). All of the males also had increased GGT and ALP and 3/4 had high AST levels. Two male and two females in the high dose group had high levels of other serum indicators of liver function/toxicity (e.g., SDH, total cholesterol, total bilirubin, or direct bilirubin). The male from the 30 mg/kg dose group which had elevated ALT and ALP at week 1 still exhibited this sign at week 4, though the female from this group did not. During week 6, only the high dose and control dogs had serum clinical chemistry measurements. The high dose dogs (all males and 2/4 females) continued to exhibit elevations in one or more serum clinical chemistry measurements associated with liver function/toxicity (especially ALT, GGT, and ALP). Increases in total and direct bilirubin and SDH were particularly great in a female animal from the 120/60 mg/kg group that was sacrificed a few days later in moribund condition. By week 8, one or more of the serum indicators of liver function/toxicity was still elevated in 2 high dose males and 1 high dose females (particularly ALT, GGT, and ALP), but the levels of these indicators were lower than the week 4 and 6 measurements. The 30 mg/kg male still exhibited elevations in ALT and ALP during week 8. At week 13, elevated ALT levels were observed in all of the males and 1 female from the 120/60 mg/kg dose group. The female and one male also had elevated ALP. In the 30 mg/kg dose group, one male (as had been observed in previous weeks) and one female had slightly elevated ALT and ALP. Drug-related changes in these serum indicators were not observed at 5 mg/kg.

Liver weight was not affected by gemifloxacin treatment. Gross lesions were not observed at necropsy. Microscopic evaluation of the liver revealed minimal to slight single cell vacuolar degeneration in all animals from the 120/60 mg/kg group and in 1 male and 1 female from the 30 mg/kg group. Minimal to slight cholangitis/pericholangitis was observed in all of the 120/60 mg/kg males and 1 male at 30 mg/kg. Moderate cholangitis/pericholangitis with amorphous biliary deposits was seen in 1 female from the high dose group that survived for the entire dosing period. Histopathology data from the high dose female that was sacrificed early revealed severe vacuolation of hepatocytes, amorphous biliary deposits, and biliary proliferation. The NOEL for liver changes was 5 mg/kg.

Plasma concentrations and AUC values for both enantiomers of gemifloxacin were similar. Quantifiable levels of gemifloxacin were generally present in dogs from the 5 mg/kg group for 8-12 hours after dosing and for the entire 24 hour sampling period for the dogs in the 30 mg/kg and 120/60 mg/kg dose groups. C_{max} occurred 0.5-4 hours after dosing. There was relatively high variability in the plasma concentration measurements between dogs, as can be observed in the SD values. C_{max} and AUC rose with ascending doses, but the increases were not strictly dose proportional.

C_{max} and AUC Values (average ± SD) for the (+) and (-) Enantiomers of Gemifloxacin In Dogs After Oral Administration

	5 mg/kg		30 mg/kg		120/60 mg/kg*	
	M	F	M	F	M	F

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Day 1	Cmax (g/ml)	+	0.0703 ± 0.037	0.0422 ± 0.012	0.636 ± 0.597	0.625 ± 0.557	2.06 ± 0.92	0.906 ± 0.440
		-	0.0770 ± 0.037	0.0459 ± 0.011	0.743 ± 0.649	0.708 ± 0.619	2.29 ± 1.06	1.10 ± 0.542
		T	0.147 ± 0.074	0.0881 ± 0.022	1.38 ± 1.25	1.33 ± 1.18	4.35 ± 1.98	2.01 ± 0.982
	AUC _{0-t} (gh/ml)	+	0.329 ± 0.135	0.210 ± 0.044	4.38 ± 2.23	3.47 ± 1.97	12.3 ± 1.90	7.66 ± 2.61
		-	0.387 ± 0.142	0.236 ± 0.059	5.07 ± 2.47	3.98 ± 2.42	14.1 ± 2.09	9.04 ± 3.07
		T	0.716 0.275	0.446 ± 0.102	9.45 ± 4.69	7.45 ± 4.39	26.4 ± 3.99	16.7 ± 5.67
Week 4	Cmax (g/ml)	+	0.0951 ± 0.049	0.0552 ± 0.068	1.01 ± 0.789	0.678 ± 0.632	1.42 ± 1.13	1.53 ± 0.904
		-	0.104 ± 0.050	0.0659 ± 0.079	1.07 ± 0.808	0.714 ± 0.617	1.56 ± 1.19	1.65 ± 0.997
		T	0.199 ± 0.099	0.121 ± 0.147	2.08 ± 1.60	1.39 ± 1.25	2.98 ± 2.32	3.18 ± 1.90
	AUC _{0-t} (gh/ml)	+	0.397 ± 0.168	0.263 ± 0.274	5.12 ± 2.50	3.79 ± 1.72	13.3 ± 6.55	14.4 ± 9.84
		-	0.466 ± 0.187	0.355 ± 0.399	5.62 ± 2.65	4.14 ± 1.69	14.1 ± 6.62	14.9 ± 9.88
		T	0.863 ± 0.355	0.618 ± 0.672	10.7 ± 5.15	7.92 ± 3.41	27.4 ± 13.2	29.4 ± 19.6
Week 13	Cmax (g/ml)	+	0.142 ± 0.092	0.0898 ± 0.056	0.544 ± 0.334	0.426 ± 0.152	2.06 ± 1.52	1.15 ± 0.772
		-	0.148 ± 0.088	0.0986 ± 0.056	0.630 ± 0.375	0.494 ± 0.165	2.26 ± 1.57	1.31 ± 0.863
		T	0.290 ± 0.180	0.188 ± 0.112	1.17 ± 0.709	0.919 ± 0.317	4.32 ± 3.09	2.46 ± 1.64
	AUC _{0-t} (gh/ml)	+	0.699 ± 0.377	0.396 ± 0.210	3.29 ± 1.45	3.86 ± 1.16	6.05 ± 2.42	6.27 ± 1.60
		-	0.788 ± 0.413	0.436 ± 0.219	3.71 ± 1.57	4.28 ± 1.24	6.71 ± 2.41	7.15 ± 1.77
		T	1.49 ± 0.789	0.832 ± 0.429	7.00 ± 3.02	8.14 ± 2.39	12.8 ± 4.83	13.4 ± 3.37

*The 120 mg/kg dose was lowered to 60 mg/kg on day 39

T = Total

The high dose used in this 13 week oral dog study, 120 mg/kg, was associated with vomiting, reduced food consumption and body weight gain and mortality. It was

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reduced to 60 mg/kg during week 5 of dosing. Vomiting was seen at a lower incidence with less frequency at 30 mg/kg. Serum clinical chemistry measurements indicative of liver function/toxicity were increased in several dogs from the 120 mg/kg group and in one dog at 30 mg/kg as early as week 1 of treatment. Although the value for many of these parameters fell in the surviving high dose dogs after the dose was lowered to 60 mg/kg, several of the measurements were still higher than baseline and control at the end of the study. Microscopic evaluation of the liver revealed minimal to slight single cell vacuolar degeneration in all animals from the 120/60 mg/kg group and in 1 male and 1 female from the 30 mg/kg group. Minimal to slight choiangitis/pericholangitis was observed in all of the 120/60 mg/kg males and 1 male at 30 mg/kg. Moderate cholangitis/pericholangitis with amorphous biliary deposits was seen in 1 female from the high dose group. Histopathology data from the high dose female that was sacrificed early revealed severe vacuolation of hepatocytes, amorphous biliary deposits, and biliary proliferation. The NOAEL for gemifloxacin was 5 mg/kg.

SB 265805: 26-Week Oral (Capsule Administration) Toxicity Study in the Dog (SB Document No. SB-265805/RSD-100X0R/2; Protocol No. G98639; Study Code 802/488)

Report dated 9/23/99, amended 10/19/99, and GLP

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Animals: Beagle dogs 10-12 months old and 7.40-12.40 kg (males) or 6.45-10.70 kg (females) on the first day of dosing, housed in pairs by sex and treatment group, 4/sex per dose group

Diet: Dog Maintenance Diet (400 g/dog) was offered daily and tap water was available *ad libitum*.

Drug Dose and Route of Administration: Gemifloxacin mesylate (Batch No. FNS-A-08C) was placed into gelatin capsules and administered orally once per day at dose levels of 0 (empty capsules), 2, 10, and 60 mg/kg/day (based on the salt).

Length and Conduct of Study: Drug was administered daily for 26 weeks with dogs receiving the final dose on the day before sacrifice.

The dogs were checked for viability twice daily. Clinical signs were recorded each day before administration of drug, after the dose was given, and 1-4 hours after dosing. The animals were weighed daily during the week prior to the initiation of dosing and during the first week of dosing, then once per week. Food consumption was monitored daily, but calculated as g/dog per week. Ophthalmic examinations (direct and

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indirect) were performed prior to the initiation of dosing and during week 26. Blood samples (for the determination of hematologic and clinical chemistry parameters) were drawn before the period of drug treatment and during weeks 1, 4, 8, 13, 17 (results not reported due to equipment failure), 18 (replacement sample for week 17), 21, and 26.

Blood for toxicokinetic evaluation was drawn from each dog on day 1 and during weeks 4 and 26 of the study 0.5, 1, 2, 4, 8, 12, and 24 hours after dosing. Plasma levels of individual gemifloxacin enantiomers were measured using _____ with a lower limit of quantification of _____ ng/ml.

In addition to the tissues recorded on the histopathology checklist, the elbow and stifle joints and a lumbar vertebra were harvested and grossly examined. Only the liver, pancreas, and sternum (with marrow) were examined microscopically.

Results: One male in the 60 mg/kg group was sacrificed in moribund condition (poor food consumption, thin, pale mucosa, dehydration) during week 3 of the study. Necropsy (including microscopic examination) revealed that the dog had pneumonia. This condition was considered the cause of mortality and was not ascribed to drug treatment.

The only clinical sign that appeared related to gemifloxacin was occasional vomiting in some of the 10 and 60 mg/kg dogs. Gemifloxacin administration did not appear to have an effect on body weight gain or food consumption. Ophthalmic examination did not reveal any lesions that appeared to be drug-related.

There were no differences in hematologic parameters between the treatment groups. The only changes in serum chemistry values that appeared to be related to gemifloxacin treatment were increases (2-14 times pretreatment values in males and 1.2-4 times pretreatment values in females) in ALT in the 60 mg/kg dogs that became most apparent after 18 weeks of dosing. Both ALT and ALP were elevated in one high dose female beginning after the first week of treatment; the high ALT did not persist for the entire study (it fell, then rose again), but the high ALP did.

Organ weights did not appear to have been affected by gemifloxacin treatment. Gross lesions were not observed at necropsy. Microscopic evaluation of the liver revealed minimal single cell vacuolar degeneration in 2/3 males and 3/4 females from the 60 mg/kg group. Mild cholangitis/pericholangitis was observed in 1/3 males and 1/4 females at 60 mg/kg. The NOEL for liver changes was 10 mg/kg.

Quantifiable levels of gemifloxacin were generally present in dogs from the 2 mg/kg group for 8-12 hours after dosing and for the entire 24 hour sampling period for the dogs in the 10 mg/kg and 60 mg/kg dose groups. C_{max} generally occurred 0.5-4 hours after dosing. There was relatively high variability in the plasma concentration measurements between dogs, as can be observed in the SD values. C_{max} and AUC rose with ascending doses and the increases were approximately dose proportional. There did not appear to be accumulation of gemifloxacin in the dogs between weeks 4 and 26 of treatment.

**C_{max} and AUC Values (average ± SD) for Gemifloxacin In Dogs
After Oral Administration**

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		2 mg/kg		10 mg/kg		60 mg/kg	
		M	F	M	F	M	F
Week 4	C _{max} (g/ml)	0.106 ± 0.026	0.064 ± 0.051	0.507 ± 0.413	0.317 ± 0.310	4.83 ± 3.08	2.00 ± 1.27
	AUC _{0-t} (gh/ml)	0.358 ± 0.097	0.247 ± 0.142	2.58 ± 1.38	1.61 ± 0.702	18.7 ± 9.01	11.3 ± 8.31
Week 26	C _{max} (g/ml)	0.154 ± 0.076	0.140 ± 0.077	0.506 ± 0.230	0.773 ± 0.581	2.54 ± 1.32	3.57 ± 2.44
	AUC _{0-t} (gh/ml)	0.745 ± 0.339	0.709 ± 0.429	2.75 ± 0.599	3.31 ± 2.02	13.1 ± 3.96	14.7 ± 4.16

Oral doses of gemifloxacin were not associated with clinical signs of toxicity other than occasional vomiting at 10 and 60 mg/kg which may have been drug-related. Microscopic evaluation of the liver revealed minimal single cell vacuolar degeneration in 2/3 males and 3/4 females from the 60 mg/kg group. Mild cholangitis/pericholangitis was observed in 1/3 males and 1/4 females at 60 mg/kg. Increases in serum ALT were associated with these liver changes. The NOAEL for gemifloxacin was 10 mg/kg in this 26 week oral dog toxicity study.

SB 265805: 14-Day Intravenous Dose Toxicity Study in Male Dogs (SB Document No. SB-265805/RSD-100TT5/1; Study No. G98542)

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Report dated 12/16/98, UK, US, and OECD GLP

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Animals: Male beagle dogs _____, 10-14 months old and 9.4-14.0 kg on the first day of dosing, housed in groups of 3 according to treatment, 3 per dose group

Diet: _____ Dog Maintenance Diet (400 g/dog) was offered daily after dosing and filtered tap water was available *ad libitum*.

Drug Dose and Route of Administration: Gemifloxacin mesylate (Batch No. FNS-A-03C) was dissolved in 0.9% NaCl and administered by IV infusion over 30 minutes at a dose volume of 5 ml/kg. The dose levels were 0 (vehicle), 3, 10, and 30 mg/kg/day (based on free drug). The 30 mg/kg dose was reduced to 20 mg/kg beginning on day 3 due to the severity of the clinical signs (apparently histamine-related) observed at the higher dose.

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Length and Conduct of Study: The dogs were infused with drug or vehicle once daily for 14 days, then sacrificed on the day after the final dose was given.

The dogs were checked for viability twice daily. Clinical signs were recorded each day before infusion of drug, after the dose was given, then 15-30 minutes and 1-4 hours after dosing. The animals were weighed daily. Food consumption was monitored daily. Ophthalmic examinations (indirect) were performed prior to the initiation of dosing and on day 10 of dosing. Blood samples (for the determination of hematologic and clinical chemistry parameters) were drawn before the period of drug treatment and on day 10 of dosing. Urine was obtained from the bladder of each dog at necropsy.

EKGs were recorded for each dog prior to the initiation of dosing and on days 2 and 9 of treatment. During the dosing period, the EKGs were taken prior to the initiation of that day's infusion, at the end of infusion, and approximately 1 hour after the end of the infusion.

Blood for toxicokinetic evaluation was drawn from each dog on days 1 and 13 of dosing 2 minutes prior to the end of infusion and 10 and 20 minutes, 1, 2, 5, 10, and 24 hours after infusions were completed. Plasma levels of individual gemifloxacin enantiomers were measured using _____ with a lower limit of quantification of _____ ng/ml.

In addition to the tissues recorded on the histopathology checklist, the elbow, hip, shoulder, and stifle joints and a lumbar vertebra were harvested and grossly examined. Several tissues were not examined microscopically including the cecum, larynx, tonsils, mammary gland, rectum, tongue, joints, and vertebrae.

Results: No unscheduled deaths occurred during the study. On the first 2 days of infusion, the following clinical signs were observed in the 30 mg/kg dogs: marked reddening of ears, marked ear and foot swelling, moderate facial swelling, ears felt moderately warm to touch, slight tremor, head shaking, lip licking, reddening of face and body, and body extremities felt hot to touch. One of the three dogs in this group became agitated during the first infusion, which was stopped until the animal relaxed, then completed. During the second infusion, another dog in this group became agitated and received only 75% of the intended dose. Based on the severity of these clinical signs, the investigators lowered the 30 mg/kg dose to 20 mg/kg on day 3 of treatment. Similar clinical signs were observed at 20 mg/kg, but at lower severity and incidence. The 20 mg/kg dose was administered and tolerated for the remainder of the study. During infusion of 10 mg/kg, clinical signs were generally considered "mild" and included reddening of the ears, ears warm to touch, facial swelling, and lip licking. On one occasion (day 7 of dosing), the clinical signs were observed at greater incidence with a greater severity and they also included reddening of the body, slight tremor, and head shaking. Examination of the dosing syringe on this day showed that the drug appeared to have come out of solution on this occasion and the investigators thought that there may have been a connection between that and the increased incidence and severity of the clinical signs. At 3 mg/kg/day, the only signs observed occurred on day 2 of infusion in 2/3 dogs and included minimal reddening and swelling of ears, ears warm to touch, and lip licking. These clinical signs usually began about 10 minutes after the start of

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infusion; peaked near the end of the infusion, and were gone within 90 minutes after dosing was over. Clinical signs such as these have been observed previously in dogs treated intravenously with other fluoroquinolones and are associated with histamine release.

Gemifloxacin administration did not appear to have an effect on body weight gain or food consumption. Changes in heart rate or ECG were not observed. Ophthalmic examination did not reveal any lesions that appeared to be drug-related.

There were no differences in hematologic parameters between the treatment groups. Urinalysis (pH, protein, glucose) did not demonstrate drug-related changes. Adrenal and prostate weights in the gemifloxacin-treated dogs tended to be higher than controls, but the differences were not statistically significant and not dose-related. Thymus weights (both absolute and relative) were significantly lower in the gemifloxacin-treated dogs than controls, though the reductions were not dose-related. There were no microscopic findings in these tissues, so the toxicologic importance is questionable. The investigators theorized that the reduction in thymus weights could be related to stress, but the reviewer is uncertain as to why the dogs in the lowest gemifloxacin dose group would be more stressed than controls since they did not experience clinical signs during the majority of the study.

There was a small increase in ALT in one dog in the 3 mg/kg group (from 34 to 74 IU/l, baseline to day 10), but no histopathologic correlate was observed, so the increase is likely to have been coincident and not related to gemifloxacin. One dog in the 20 mg/kg group had an increase in ALT from 30 IU/l at baseline to 98 IU/l on day 10. Microscopic findings in the liver of this dog included minimal focal pericholangitis and minimal proliferation of bile duct elements. A second dog in the high dose group also showed minimal proliferation of bile duct elements. These liver changes are believed to be gemifloxacin-related. The report mentions a previous study where Raman microspectrometry indicated that drug-related material was present in the biliary system of dogs. Pericholangitis was also observed in the earlier study and considered by the investigators to be secondary to the precipitate.

Slight to marked reddening of the injection sites was seen in all groups. Microscopic examination revealed minimal to marked inflammation and other tissue changes such as hemorrhage, vasculitis (associated with needle tract), necrosis, fibroplasia, and thrombosis at the injection sites. However, these changes were observed in all treatment groups and neither severity nor incidence appeared related to dose.

Quantifiable levels of gemifloxacin were generally present in plasma for the entire 24 hour sampling period. C_{max} occurred just prior to the end of infusion. The variability in the plasma concentration measurements between dogs was relatively low. C_{max} and AUC rose with ascending doses and the increases were approximately dose proportional. There did not appear to be accumulation of gemifloxacin in the dogs between days 1 and 13 of treatment.

**C_{max} and AUC Values (average ± SD) for Gemifloxacin In Male Dogs
After Intravenous Administration**

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		3 mg/kg	10 mg/kg	30/20 mg/kg
Day 1	C _{max} (g/ml)	0.891 ± 0.13	2.31 ± 0.44	11.54 ± 4.56
	AUC _{0-t} (gh/ml)	2.49 ± 0.82	9.20 ± 0.40	25.26 ± 4.81
Day 13	C _{max} (g/ml)	0.706 ± 0.21	1.85 ± 0.22	5.42 ± 2.15
	AUC _{0-t} (gh/ml)	2.19 ± 0.48	9.39 ± 1.02	16.30 ± 1.91

IV administration of gemifloxacin was associated with clinical signs including red ears, face, and body; swelling of ears, feet, and face; warm/hot ears and extremities, slight tremor, head shaking, lip licking, and agitation. The incidence and severity of these signs were dose-related and their intensity at 30 mg/kg resulted in a reduction to 20 mg/kg on day 3 of administration. The clinical signs were much less severe and observed less frequently in the 10 mg/kg dose group. At 3 mg/kg, clinical signs (considered minimal in severity) were only observed on day 2 of administration in 2/3 dogs and included red, swollen, warm ears, and lip licking. Signs such as these have been observed in dogs when other fluoroquinolones were administered intravenously and appear to be related to histamine release. Microscopic findings in the liver of a dog from the 20 mg/kg group included minimal focal pericholangitis and minimal proliferation of bile duct elements. This animal exhibited an increase in serum ALT (about 3 times baseline). A second dog in this high dose group also showed minimal proliferation of bile duct elements. The NOAEL for gemifloxacin was 10 mg/kg in this 14 day IV dog toxicity study, based upon histopathology.

SB 265805: 28-Day Intravenous (1-Hour Infusion) Dose Toxicity Study in Dogs (SB Document No. SB-265805/RSD-100ZRV/2; Study Code 802/519)

Report dated 5/27/99, amended 10/15/99, and US GLP

Vol. 1.5.017

Animals: Beagle dogs, 11-13 months old, 8.4-13.0 kg (males) and 8.4-11.0 kg (females) on the first day of dosing, housed singly, 3/sex per dose group
Diet: Diet was offered daily after dosing and filtered tap water was available *ad libitum*.

Drug Dose and Route of Administration: Gemifloxacin mesylate (Batch No. FNS-A-08C) was dissolved in 0.9% NaCl and administered by IV infusion over 1 hour at a dose volume of 5 ml/kg. The dose levels were 0 (vehicle), 2, 10, and 20 mg/kg/day (based on free drug). An indwelling catheter was implanted into the anterior vena cava and kept patent between test article infusions with the continuous infusion of saline at 1 ml/hr.

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Length and Conduct of Study: The dogs were infused with drug or vehicle once daily for 28 days, then sacrificed on the day after the final dose was given.

The dogs were checked for viability twice daily. Clinical signs were recorded each day before infusion of drug, after the dose was given, and 1-5 hours after dosing. Starting on day 6 of treatment, clinical signs were also recorded 30-45 minutes after the initiation of infusion. The animals were weighed twice weekly. Food consumption was monitored daily. Ophthalmic examinations (indirect) were performed prior to the initiation of dosing and during week 4 of treatment. Blood samples (for the determination of hematologic and clinical chemistry parameters) were drawn before the period of drug treatment and during weeks 1 and 4 of dosing after an overnight fast and prior to that day's drug infusion. Urine was obtained from the bladder of each dog at necropsy.

EKGs were recorded for each dog prior to the initiation of dosing and during weeks 1 and 4 of treatment. During the dosing period, the EKGs were taken prior to the initiation of that day's infusion and just before the end of infusion using standard limb leads I, II, and III.

Blood for toxicokinetic evaluation was drawn from each dog on days 1 and 28 of dosing just prior to the end of infusion and 1.5, 2, 5, 8, 12 and 24 hours after the start of infusion. Plasma levels of individual gemifloxacin enantiomers were measured using _____ with a lower limit of quantification of ---ng/ml .

In addition to the tissues recorded on the histopathology checklist, the elbow, hip, shoulder, and stifle joints and a lumbar vertebra were harvested and grossly examined. Several tissues were not examined microscopically including the bone marrow smears, cecum, cervix, larynx, tonsils, mandibular lymph nodes, rectum, tongue, joints, and vertebrae. Tissue samples from the liver, kidney, and injection sites were examined under polarized light for the presence of crystals.

Results: One male from the 10 mg/kg group was sacrificed in poor condition on day 11. This dog had catheter-related severe swelling and inflammation of the chest, neck, and left shoulder and a soft swelling in the left axilla, observed on day 8 of infusion about 4 hours after dosing. The animal had a fever and was lethargic. Infusions were suspended after day 8 and the animal was sacrificed 3 days later when its condition did not improve. Microscopic evaluation of the catheter site revealed severe perivasculitis and a marked thrombus containing crystals.

Clinical signs associated with the infusion were seen in 2 females from the 20 mg/kg dose group. These included swelling of forelimbs, ears, and face and redness of ocular membranes. Unsteady gait was also observed in one dog. These signs were generally gone by 4 hours following the end of infusion and were likely secondary to gemifloxacin-related histamine release.

Body weight gain and food consumption were not affected by gemifloxacin administration. No drug-related effects on heart rate or ECG were observed. Ophthalmic changes related to gemifloxacin were not seen in any treatment group.

Gemifloxacin treatment was not associated with changes in hematologic parameters. Plasma AST levels were increased about 2-3 fold above baseline in week 4

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in all males from the 20 mg/kg group and ALT levels were increased about 6-8 fold in the same dogs. All females from the 10 mg/kg group had increased ALT levels (about 2-10 fold), as did 2/3 females from the 20 mg/kg group (about 2-3 fold). One of these 20 mg/kg females exhibited increased ALT during week 1. Globulin levels were reduced by about 30% compared to baseline in 1 male and 2 females from the 20 mg/kg group. SDH activity was increased by 2-3 fold in one 10 mg/kg female and 1 male and 1 female at 20 mg/kg. Urinalysis did not reveal any drug-related changes.

Microscopic examination revealed drug-related changes in the liver. Minimal to slight pericholangitis was observed in all 20 mg/kg dogs and 2 females at 10 mg/kg. Brown pigment was observed in the liver of 2/3 females and all males in the high dose group. Minimal bile duct proliferation was seen only in the males at 20 mg/kg. A few "degenerated" hepatocytes (said to have a "ballooned" appearance) were seen in 1-2 dogs in the control and low dose gemifloxacin groups, but hepatocytes with this sort of appearance were observed more frequently and in larger numbers (forming aggregates) in several dogs from the 10 and 20 mg/kg dose groups. Most changes at the injection site were not associated specifically with gemifloxacin treatment. Two females in the 10 mg/kg group and 1 in the 2 mg/kg group had crystals associated with a thrombus, but this was not observed in the females from the 20 mg/kg group or in any males other than the dog from the 10 mg/kg group that was sacrificed early.

Plasma concentrations and AUC values for both enantiomers of gemifloxacin were similar with the (-) isomer usually slightly higher. Quantifiable levels of gemifloxacin were generally present in dogs from the 2 mg/kg group for up to 12 hours after dosing and for the entire 24 hour sampling period for the dogs in the 10 mg/kg and 20 mg/kg dose groups. Cmax and AUC rose with ascending doses in a generally dose proportional manner. There was no evidence of accumulation over the course of the study.

Cmax and AUC Values (average ± SD) for the (+) and (-) Enantiomers of Gemifloxacin In Dogs After Intravenous Administration

			2 mg/kg		10 mg/kg		20 mg/kg	
			M	F	M	F	M	F
Day 1	Cmax (g/ml)	+	0.160 ± 0.03	0.182 ± -0.02	0.975 ± 0.13	0.903 ± 0.15	1.99 ± 0.28	2.01 ± 0.19
		-	0.170 ± 0.03	0.188 ± 0.01	1.02 ± 0.12	0.963 ± 0.15	2.09 ± 0.24	2.05 ± 0.15
		T	0.330 ± 0.05	0.369 ± 0.03	1.99 ± 0.25	1.87 ± 0.30	4.09 ± 0.53	4.07 ± 0.34
	AUC _{0-t} (gh/ml)	+	0.728 ± 0.03	0.756 ± 0.09	4.90 ± 0.32	4.55 ± 1.31	8.45 ± 0.87	10.6 ± 1.05
		-	0.785 ± 0.04	0.808 ± 0.09	5.31 ± 0.30	4.87 ± 1.36	8.84 ± 0.66	11.2 ± 1.37

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		T	1.51 ± 0.07	1.56 ± 0.18	10.2 ± 0.61	9.41 ± 2.67	17.3 ± 1.50	21.8 ± 2.42
Day 28	C _{max} (g/ml)	+	0.161 ± 0.02	0.170 ± 0.02	0.890 ± 0.07	0.843 ± 0.12	1.85 ± 0.16	1.89 ± 0.17
		-	0.167 ± 0.02	0.174 ± 0.02	0.933 ± 0.07	0.891 ± 0.11	1.97 ± 0.16	1.98 ± 0.14
		T	0.328 ± 0.04	0.344 ± 0.04	1.82 ± 0.15	1.73 ± 0.23	3.82 ± 0.32	3.87 ± 0.32
	AUC _{0-t} (gh/ml)	+	1.13 ± 0.76	0.791 ± 0.15	4.95 ± 0.13	4.74 ± 1.18	10.1 ± 0.74	10.9 ± 2.13
		-	1.15 ± 0.68	0.833 ± 0.13	5.27 ± 0.30	5.03 ± 1.10	10.7 ± 0.43	11.4 ± 2.14
		T	2.28 ± 1.44	1.62 ± 0.28	10.2 ± 0.43	9.77 ± 2.27	20.8 ± 1.16	22.3 ± 4.27

T = Total

Histamine-related clinical signs (swelling of forelimbs, ears, and face and redness of ocular membranes) were observed after 20 mg/kg gemifloxacin infusion in 2/6 dogs (both female). Increases in plasma liver enzymes and microscopic changes in the liver (hepatocyte degeneration, minimal to slight pericholangitis, bile duct proliferation, presence of brown pigment) were observed in several dogs from the 10 and 20 mg/kg dose groups. The NOAEL for gemifloxacin given intravenously over one hour each day for 28 days to the dog was 2 mg/kg.

REPRODUCTIVE TOXICOLOGY

SB 265805: Oral (Gavage) Rat Developmental Toxicity Dose Ranging Study (SB Document No. SB-265805/RSD-100NT6/1; Project Code _____)

Report dated 6/23/97, — and ———JLP

Vol. 1.5.022

Animals: Timed-mated female Sprague-Dawley rats, 10-12 weeks old, 5 per dose group

Diet: SQC Rat and Mouse No. 3 Breeder pelleted diet and tap water were provided *ad libitum*.

Drug Dose and Route of Administration: Gemifloxacin (Lot No. LB20304a-02R1P1) was dissolved in 0.9% aqueous saline and administered daily (10 ml/kg) from days 6-17 of gestation by oral gavage at doses of 0 (vehicle control), 30, 90, 270, and 750 mg/kg

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(not clear whether dose was based on mesylate salt or free drug).

Length and Conduct of Study: The rats were sacrificed on day 20 of gestation. Corpora lutea, fetuses, and implantation sites were counted, and fetuses were examined for external abnormalities. The fetuses and placentae were weighed.

Appearance and behavior of the rats were monitored twice daily and body weight was recorded daily from days 5-17 of gestation and on day 20 of gestation. Food consumption was measured at these intervals during gestation: days 5-6, 6-9, 9-12, 12-15, 15-17, and 17-20.

Results: No unscheduled deaths occurred during the study. The rats in the 750 mg/kg group became "difficult to dose" during days 10-16 of gestation. Increased fecal output was observed in 3/5 and 4/5 females in the 270 and 750 mg/kg dose groups, respectively. Body weight gain in the 750 mg/kg group was significantly reduced compared to control between days 15-17 of gestation (the end of the dosing period). Food consumption in the 250 and 750 mg/kg groups was significantly reduced compared to control during the first part of the dosing period, days 6-9. Food consumption in these groups was similar to control for the remainder of the study.

Gross necropsy of the dams revealed no gemifloxacin-related changes. One rat each in the 30 and 750 mg/kg groups was not pregnant. The mean number of corpora lutea (14.2-17.4) per female did not differ significantly between groups. Treatment with gemifloxacin at doses up to 750 mg/kg did not alter the mean number of implantation sites (11.2-15.3) and it did not cause increased pre- or postimplantation loss or reduce fetal viability. In control rats, preimplantation loss was 13.2% and postimplantation loss was 7.5%. In drug treated rats, postimplantation loss ranged from 3.6-9.8%. Mean preimplantation loss in the 90 mg/kg group was significantly higher than control, but it was primarily due to one female which had preimplantation loss of 94%. The 2 higher gemifloxacin dose groups did not demonstrate increased preimplantation loss compared to control, so the loss at 90 mg/kg was not considered drug-related.

Although fetal viability was not affected by drug treatment, mean fetal (3.78 ± 0.31 vs. 3.09 ± 0.43) and placental weights (0.60 ± 0.06 vs. 0.42 ± 0.06) in the 750 mg/kg dose group were significantly lower than control, perhaps indicative of fetotoxicity at the highest gemifloxacin dose. No external fetal abnormalities were observed.

Based on the results of this study, the doses chosen for the dams for the subsequent pivotal fertility/early embryonic development study were 30, 270, and 750 mg/kg.

SB 265805: Rat Oral (Gavage) Fertility and Embryonic Development Study (SB Document No. SB-265805/RSD-100NT5/2; Contract Study Code LGM/2/R)

Report dated 3/26/98, amended 10/20/99, and ——— GLP

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Animals: Male and female Sprague-Dawley rats, 7-8 weeks old (males) and 11-13 weeks old (females) at the initiation of dosing, 25 per dose group for main reproduction study and 9 satellite females per gemifloxacin group for toxicokinetics, housed in groups of 3-5 by sex prior to mating and post mating for the males (females individually housed after mating)

Diet: SQC Rat and Mouse No. 3 Breeder pelleted diet and tap water were provided *ad libitum*.

Drug Dose and Route of Administration: Gemifloxacin (Lot No. LB20304a-02R1P1) was dissolved in 0.9% aqueous saline and administered daily (10 ml/kg) by oral gavage. Males received doses of 0 (vehicle control), 30, 90, and 270 mg/kg starting at least 63 days prior to mating and continuing until the day before necropsy. Females received doses of 0 (vehicle control), 30, 270, and 750 mg/kg starting 14 days before mating and continuing through day 17 of gestation. These doses were based on the mesylate salt and were equivalent to 24, 216, and 600 mg/kg/day for the gemifloxacin free base for the 30, 270, and 750 mg/kg doses, respectively. (mesylate salt) equivalent to 24, 216, and 600 mg/kg/day for the gemifloxacin free base. Previous toxicity studies have demonstrated crystal nephropathy in male and female rats after one month of dosing at 750 mg/kg. This information, along with the range finding study above, was used to choose the doses for the current study.

Blood samples were drawn from the last 3 females in each group assigned for the main reproduction study prior to dosing and 90 minutes and 150 minutes after the first dose was given. For toxicokinetic evaluation, blood samples were drawn from the satellite females on days 6 and 17 of gestation prior to dosing, then 10 and 30 minutes and 1, 1.5, 2.5, 4, 8, and 12 hours after gemifloxacin was administered. The results of the toxicokinetic study were reported separately (see below).

Length and Conduct of Study: Vaginal smears were taken daily from the female rats daily during the 2 weeks immediately preceding the mating period to monitor the estrus cycle. Male and female rats were paired 1 to 1 according to dose group (control, low, mid, and high). The day on which sperm was detected in the vaginal smear was considered day 0 of gestation. Four pairs failed to mate after 10 days of cohabitation and the females were paired with another male from the same group that had mated previously with another female. The males that initially failed to mate were cohabited with a test-mate female to determine their ability to impregnate. The test mate females were sacrificed on day 14 of gestation and their pregnancy status assessed.

The males were sacrificed at the end of mating and the female rats were sacrificed on day 20 of gestation. Testes and epididymides were weighed prior to fixing in Bouin's fluid. Corpora lutea, fetuses, and implantation sites were counted, and fetuses were examined for external abnormalities. The fetuses and placentae were weighed. Half of

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the fetuses from each litter were fixed in Bouin's fluid so that they could be examined for visceral abnormalities using a combined sectioning, dissection technique. The other half were fixed in 70% alcohol, examined via microdissection, eviscerated, then cleared and stained with Alizarin red S for skeletal examination.

Appearance and behavior of the rats were monitored twice daily. For males, body weight was recorded twice weekly and for females, although it was measured twice weekly prior to mating and daily during gestation, body weight was reported only on days 1, 4, 8, 11, and 15 prior to mating and on days 0, 6, 12, 17, and 20 of gestation. Food consumption was measured weekly prior to the mating period at these intervals during gestation: days 0-6, 6-12, 12-17, and 16-20.

Results: One female in the 750 mg/kg group was sacrificed in moribund condition (irregular breathing, unsteady gait, hypoactivity) near the end of gestation. The animal was pregnant and carried live fetuses. The investigators felt it unlikely that the death was treatment-related because none of the other animals in this dose group exhibited similar symptoms.

The only clinical sign observed during the study that appeared related to gemifloxacin treatment was "noisy breathing" which was observed in several rats from the 270 (both males and females) and 750 (contained females only) mg/kg dose groups.

No significant effects on body weight gain were observed during the study. Food consumption was not affected in the males or females at doses ≤ 270 mg/kg. A slight, but statistically significant decrease in food consumption was observed in the 750 mg/kg females during the first week of treatment. After this initial decrease, however, food consumption in this group was similar to controls for the remainder of the study.

Gemifloxacin did not appear to have any effects on the estrous cycle, the copulation index, or the fertility index. In the mid and high dose groups, the pre-coital interval was slightly longer than the control group (though this increase was not statistically significant) because the rats did not mate at the first opportunity. The majority mated at the next opportunity.

Necropsy did not reveal any gross treatment-related findings in either the male or female rats. Testicular weights in the treated males did not differ from controls. Of the 25 females mated in each group, 21 control animals were pregnant and 23 animals in each gemifloxacin group were pregnant. The litter data from one female in the 270 mg/kg group was not counted because the animal had not shown a sperm-positive vaginal smear and the investigators incorrectly estimated day 20 of pregnancy (upon sacrifice and cesarean section, the gestation day was apparently earlier). The litter data from the 750 mg/kg female sacrificed in moribund condition was also not counted.

Treatment of female rats with gemifloxacin at doses up to 750 mg/kg did not alter the mean number of corpora lutea (14.9-15.7) or implantation sites (13.3-14.1) and it did not cause increased pre- or postimplantation loss or reduce fetal viability. In control rats, mean preimplantation loss was 10.3% and mean postimplantation loss was 6.1%. In drug treated rats, mean preimplantation loss ranged from 9.0-13.6% and mean postimplantation loss ranged from 4.0-6.2%.

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Although fetal viability was not affected by drug treatment, mean fetal (3.68 ± 0.17 vs. 3.44 ± 0.21) and placental weights (0.59 ± 0.06 vs. 0.52 ± 0.05) in the 750 mg/kg dose group were significantly lower than control, perhaps indicative of fetotoxicity at the highest gemifloxacin dose. External and visceral examination of the fetuses did not reveal drug-related abnormalities- some visceral malformations and variations were observed in all groups, but there were no dose-dependant increases in the variety or severity of abnormalities. The incidence of decreased or incomplete ossification of several bones was higher in the high dose fetuses than controls. The investigators considered this type of observation to be a "minor abnormality". This observation is consistent with the reduced fetal weights in the high dose group and may also be related to fetotoxicity. The incidence of incomplete ossification of the occipital bone of the skull was higher in all gemifloxacin-treated fetuses than controls, but a dose-response relationship was not observed.

Skeletal Observations ("Minor Abnormalities") in Fetuses from Gemifloxacin-Treated Rats

	Control	30 mg/kg	270 mg/kg	750 mg/kg
# Litters Examined	21	23	22	22
# Litters Affected	7	6	7	13
Mean % Fetuses Affected (Total)	6.6	4.3	9.5	14.9
Specific Skeletal Observations (expressed as mean % fetuses affected)				
Caudal Vertebrae (# Centra \leq2)	0	0	0	4.5*
2nd Sternebra: Not Ossified	0	0.6	0	2.0
2nd Sternebra: Inc. Ossific.	2.9	1.7	2.1	10.5*
3rd Sternebra: Inc. Ossific.	0	1.1	0.9	2.7
5th Sternebra: Not Ossified	3.9	2.0	1.6	11.9
6th Sternebra: Not Ossified	0	0.5	0.6	2.7
Pubis: Inc. Ossific.	0	0.6	0	2.8
Metacarpal (\geq1): Not Ossified	2.0	7.1	7.3	23.4*
Inc. Ossific. of Occipital	0.7	4.1	9.2	5.7

*p<0.05 (Cochran-Armitage and Fisher's Exact Test) for individual parameter compared to control

Aside from an increased incidence of "noisy breathing" at 270 and 750 mg/kg/day and an initial reduction in food consumption at 270 mg/kg that was not sustained for the duration of the treatment period, no maternal or paternal gemifloxacin toxicity was observed. It should be noted that higher doses of drug were not used for this study as

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other studies in this species have revealed crystal nephropathy when 750 mg/kg of gemifloxacin was given for one month. Female fertility and pre- or postimplantation losses were not affected at doses up to 750 mg/kg and male fertility was not affected at doses up to 270 mg/kg. Fetotoxicity (manifested by reduced fetal and placental weights and reduced skeletal ossification at several sites) was observed at the high dose (750 mg/kg for dams and 270 mg/kg for sires).

Quantification of LB20304 in Rat Serum Samples from a Rat Oral (Gavage) Fertility and Embryonic Development Study (SB Document No. SB-265805/RSD-100TT7/1; Contract Study Code 2W001)

M. Morrison (_____)

Report dated 6/9/98, _____ and _____ GLP

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Summary: These are the results of the toxicokinetics portion of the fertility and early embryonic development study described above. The serum concentrations of gemifloxacin were measured using a validated _____ method with _____ detection (lower limit of quantification was _____ g/ml).

Mean Serum Concentrations of Gemifloxacin (g/ml) in Females Assigned to the Main Reproduction Study 1.5 and 2.5 Hours after Oral Dosing (n=3 per dose group)

	1.5 Hours	2.5 Hours
30 mg/kg	0.670	0.445
270 mg/kg	4.79	3.26
750 mg/kg	8.31	5.62

Gemifloxacin could be detected in the serum from the pregnant rats used for main reproduction study for at least 2.5 hours after oral dosing.

**Toxicokinetic Parameters for Gemifloxacin
After Daily Oral Dosing to Pregnant Female Rats**

	30 mg/kg	270 mg/kg	750 mg/kg
Day 6 of Gestation			
C _{max} (g/ml)	0.402	2.84	7.49
T _{max} (h)	0.17	0.5	0.5
AUC _{0-t} (gh/ml)*	0.845	10.8	26.5
AUC _{0-24h} (gh/ml)	Not Calculated	Not Calculated	35.5
Day 17 of Gestation			
C _{max} (g/ml)	0.429	4.55	10.9

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Tmax (h)	0.5	0.5	0.5
AUC_{0-t} (gh/ml)*	0.811	14.9	28.5
AUC_{0-24 h} (gh/ml)	Not Calculated	Not Calculated	37.6

*AUC_{0-t} where t is the last time point where the serum drug level was above the limit of quantification.

There was no evidence of accumulation between days 6 and 17 of gestation. As the rats had been dosed beginning 14 days prior to the mating period, steady state is likely to have been achieved by the time of the toxicokinetic sampling. The last time points used for the toxicokinetic study where serum gemifloxacin levels were above the limit of quantification was 4 h, 12 h, and 12 h for the 30, 270, and 750 mg/kg dose groups, respectively. The increases in C_{max} and AUC values were close to dose proportional as gemifloxacin doses were raised.

SB 265805: Oral Dose Range Study for Effects on Embryo-Fetal Development in Mice (SB Document No. SB-265805/RSD-100V0X/1; Protocol No. D97219)

K. Treinen (SmithKline Beecham, King of Prussia, PA)

Report issued 11/25/98, UK, US, and OECD GLP

Vci. 1.5.023

Animals: Male and female CD-1(ICR)BR mice (only females received gemifloxacin), 10-15 weeks old and 20-50 g at the time of mating, 20 mated females in control group and 10 mated females in each gemifloxacin dose group

Diet: Certified Rodent Chow _____ and filtered tap water were provided *ad libitum*.

Drug Dose and Route of Administration: Gemifloxacin (Batch No. 03R1P2-1-1) was dissolved in 0.9% sodium chloride and administered via oral gavage at a dose volume of 10 ml/kg. The dose groups were 0 (vehicle control), 10, 60, 150, 300, 450, 600, and 1000 mg/kg (based on free drug). Initially, the highest dose group was 300 mg/kg, but the higher dose groups were added when significant toxicity was not observed at this level.

Length and Conduct of Study: Male and female mice were cohabitated 1:1. Females were examined for vaginal plugs in the morning and afternoon. The day that a vaginal plug was observed was considered day 0 of gestation. Females were randomly assigned to dose groups as they were mated. Female mice were dosed on days 6-15 of gestation and sacrificed on day 18. The ovaries were removed and corpora lutea counted. The uterus was weighed, examined for implantation sites (ammonium sulfide staining was used as needed), and the number of resorptions and live or dead fetuses was determined.

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Placentas and fetuses were examined grossly.

For the mated females, body weights were measured on day 0 of gestation and daily from days 6-18. Food consumption was measured at the intervals days 0-6, 6-9, 9-12, 12-16, and 16-18. The mice were observed daily for viability and clinical signs.

Results: One female each from the 450 and 1000 mg/kg dose groups died during the dosing period (days 14 and 7 of gestation, respectively). Prior to death, these mice were cold to the touch, dehydrated, and had tremors; observations consistent with their moribund condition. Gross necropsy did not reveal a specific cause of death. The investigators considered the deaths related to gemifloxacin because mortality has been observed in mice at doses ≥ 400 mg/kg in other toxicity studies.

There was a dose-related decrease in body weight gain compared to controls in the mice given ≥ 450 mg/kg gemifloxacin. On day 18, the mean body weights of the 450, 600, and 1000 mg/kg mice were 11, 19, and 31% less than controls, respectively. Decreased food consumption during days 6-18 of gestation was also observed in these mice, with the percent reductions in food consumption compared to control paralleling the decreased mean body weights for each of the dose groups ≥ 450 mg/kg.

The numbers of corpora lutea and implantation sites were similar between dose groups. There was, however, an increase in resorptions (primarily early) at doses of gemifloxacin ≥ 450 mg/kg. One female each in the 450 and 600 mg/kg groups and 2 females in the 1000 mg/kg group resorbed all or the majority (e.g., 11/13 fetuses) of their litters. Mean postimplantation loss (\pm SEM) in the control group was $8.1 \pm 2.3\%$, compared to $12.2 \pm 9.2\%$, $19.4 \pm 10.3\%$, and $35.7 \pm 15.0\%$ in the 450, 600, and 1000 mg/kg dose groups. Decreased litter size (10.0, 9.7, and 7.8 for 450, 600 and 1000 mg/kg, respectively) compared to control (11.2) was observed in these dose groups.

There was a dose-related decrease in mean fetal body weight compared to control in the mice given ≥ 300 mg/kg gemifloxacin. The mean weights (\pm SEM) of male and female control fetuses were 1.40 ± 0.03 g and 1.32 ± 0.02 g. The percent decreases compared to control for male and female fetuses were 6% and 9% at 300 mg/kg, 12% and 15% at 450 mg/kg, 29% and 30% at 600 mg/kg, and 44% (for both sexes) at 1000 mg/kg. A few external malformations (e.g., open eye, bent tail, cleft palate, exencephaly) were observed in 0-2 litters per dose group, but none appeared related to gemifloxacin.

Maternal toxicity (including mortality, reduced food consumption, and reduced body weight gain) was observed at gemifloxacin doses ≥ 450 mg/kg. Increased postimplantation loss (early resorption) was also seen at gemifloxacin doses ≥ 450 mg/kg. Decreased fetal body weights were observed at doses of gemifloxacin ≥ 300 mg/kg.

SB 265805: Oral Study for Effects on Embryo-Fetal Development in Mice (SB Document No. SB-265805/RSD-100V0J/1; Protocol No. G97220)

K.A. Treinen and S. Portelli (SmithKline Beecham, King of Prussia, PA)

Report issued 9/22/98, UK, US, and OECD GLP

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Animals: Male and female CD-1(ICR)BR mice (only females received gemifloxacin), approximately 11 weeks old and 20-30 g at the time of mating, 23 mated females per dose group for the main reproduction study and 12 mated satellite females per dose group for toxicokinetics

Diet: Certified Rodent Chow _____ and filtered tap water were provided *ad libitum*.

Drug Dose and Route of Administration: Gemifloxacin (Batch No. 02R1P1 Lot 2) was dissolved in 0.9% sodium chloride and administered via oral gavage at a dose volume of 10 ml/kg. The dose groups were 0 (vehicle control), 60, 250, and 450 mg/kg (based on free drug). These doses were selected based upon the results of a range-finding study (above) that demonstrated significant maternal toxicity at doses ≥ 450 mg/kg.

Length and Conduct of Study: Male and female mice were cohabitated 1:1. Females were examined for vaginal plugs once daily. The day that a vaginal plug was observed was considered day 0 of gestation. Mated females were randomly assigned to dose groups based on body weight. Female mice were dosed on days 6-15 of gestation and sacrificed on day 18. The ovaries were removed and corpora lutea counted. The uterus was weighed, examined for implantation sites (ammonium sulfide staining was used as needed), and the number of resorptions and live or dead fetuses was determined. Placentas and fetuses were examined grossly. Live fetuses were weighed. Approximately half of the fetuses were assigned for abdominal and thoracic visceral examination using a modified Staples' technique. These fetuses were decapitated and the heads preserved in Bouin's solution and sectioned for examination. The other half of the fetuses were processed for skeletal examination including staining with Alizarin Red S.

For the mated females, body weights were measured on day 0 of gestation and daily from days 6-18. Food consumption was measured at the intervals days 0-6, 6-9, 9-12, 12-16, and 16-18. The mice were observed daily for viability and clinical signs.

For the toxicokinetic study, satellite groups of pregnant mice were dosed with gemifloxacin from days 6-10 of pregnancy. Four satellite mice per dose group had blood samples collected into EDTA 1, 3, 5, 8, 12, and 24 hours after dosing on day 10 of pregnancy. Plasma concentrations of gemifloxacin were measured using an _____ assay with _____ and a lower limit of detection of _____ ng/ml.

Results: Two control, one 60 mg/kg, and two 250 mg/kg dams were not pregnant. Two females in the 450 mg/kg group were found dead on days 13 and 16 of gestation. The investigators believed that the mortality was drug-related because deaths have occurred at gemifloxacin doses ≥ 400 mg/kg in previous studies with pregnant and

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nonpregnant mice; however, necropsy did not reveal a specific cause of death. Two females in the 60 mg/kg group had swelling around the shoulder area. One was killed in moribund condition on day 15 of gestation and the other was sacrificed as scheduled. Necropsy revealed that each of these animals had a torn esophagus resulting from gavage accidents. The swollen shoulder areas contained food which had leaked out of the esophagus.

Several mice delivered their litters early (day 17 or 18 of gestation), prior to scheduled cesarean section. This included 1 each from the control and 60 mg/kg groups and 2 from the 450 mg/kg groups. All of the pups from the 2 early 450 mg/kg litters were found dead or were cannibalized. One pup each from the control and 60 mg/kg litters were presumed cannibalized, but the rest were viable.

There were no drug-related changes in maternal body weight gain or food consumption. Clinical signs specifically related to gemifloxacin treatment were not noted. Two mice in the 60 mg/kg group entirely resorbed their litters, but the investigators did not believe this to be a drug-related effect since it did not occur in the higher dose groups.

The mean number of corpora lutea and implantation sites were similar between the treatment groups and gemifloxacin exposure did not appear to increase postimplantation loss at doses up to 450 mg/kg. The mean number of live fetuses per litter was similar in the gemifloxacin-treated animals and controls.

There was a statistically significant decrease in mean fetal body weight in the 450 mg/kg group (a 9% decrease for males and a 10% decrease for females). Gemifloxacin exposure was not associated with an increase in external or visceral abnormalities. An increase in the mean % (\pm SEM) of fetuses per litter with cervical rib compared to control was observed at 450 mg/kg ($10.79 \pm 3.81\%$ vs. $26.74 \pm 7.5\%$), but the increase was not statistically significant (Cochran-Armitage Trend Test). Delayed ossification manifested by a statistically significant increase (Cochran-Armitage Trend Test) in the mean % of fetuses per litter with one or more cervical vertebrae centra not evident was observed at 450 mg/kg (0 vs. $7.77 \pm 5.3\%$), consistent with the decreased body weight seen in these mice.

C_{max} and AUC_{0-t} rose in a linear fashion. The investigators thought it possible that C_{max} was underestimated as T_{max} was equal to the time point when the first sample was drawn. Plasma concentrations generally stayed above the level of detection for 12 hours.

**Mean Toxicokinetic Parameters in Pregnant Mice
Following Multiple Oral Doses of Gemifloxacin**

Gemifloxacin Dose	C _{max} (g/ml)	AUC _{0-t} (gh/ml)	T _{max} (h)
60 mg/kg	0.308	1.87	1.16
250 mg/kg	1.11	6.74	1.01
450 mg/kg	2.37	15.4	0.97

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No adverse effects on dams or fetuses were observed at gemifloxacin doses up to 250 mg/kg when the drug was administered orally to mice from days 6-15 of gestation. A gemifloxacin dose of 450 mg/kg was associated with maternal mortality (but not clinical signs of toxicity including reduced body weight gain or food consumption) and fetotoxicity. Mean fetal body weights at 450 mg/kg were significantly reduced compared to control and delays in skeletal observation were observed, indicative of fetal growth retardation.

SB 265805: Intravenous Maximally Tolerated Dose Study in Rabbits (SB Document No SB-265805/RSD-100Z9X/1; Protocol No. D98078)

H.M. Solomon (SmithKline Beecham, King of Prussia, PA)

Report issued 3/12/99, not GLP

Vol. 1.5.023

Animals: Female nonpregnant New Zealand White rabbits (Hra(NZW)SPF), approximately 7 months old, 3-5 kg, 2 per dose group

Diet: Approximately 125 g of Lab Rabbit Chow was provided each day and tap water was available *ad libitum*.

Drug Dose and Route of Administration: Gemifloxacin (Batch No. 02R1P1 Lot 2) was dissolved in 0.9% sodium chloride and administered once daily into the ear vein. Groups 1-3 received the infusion as a rapid bolus and Groups 4-5 received a slower injection (20 ml/min). The doses (based on free drug), concentrations of gemifloxacin solution, and dose volumes were as follows for each treatment group:

Treatment Group	Daily Dose (mg/kg)	Dose Volume (ml/kg)	Concentration (mg/ml)	Post-Dose Saline Flush (1 ml/kg)
1	30	5	6	No
2	20	1	20	No
3	20	5	4	No
4	5	5	1	Yes
	10	5	2	Yes
5	20	5	4	Yes

Length and Conduct of Study: The study plan called for the drug to be given daily for up to 14 consecutive days. On day 6 of dosing, blood samples from one rabbit each in Groups 1 and 3 were collected into EDTA 1, 10, and 30 minutes, and 1, 2, 5, 10, and 24

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hours after dosing. Gemifloxacin plasma concentrations were measured using _____ with a lower limit of quantification of _____ ng/ml.

Results: Severe injection site reactions occurred in both rabbits from groups 1-3 and in 1/2 rabbits from group 5. These animals could not be dosed for the entire 14 days planned for the study. In group 1, one rabbit was dosed for 5 days and the other was dosed for 6 days; in group 2, one animal was dosed for 3 days and the other for 4 days; in group 3, one rabbit was dosed for 5 days and the other for 7 days; and in group 5, one rabbit was dosed for 7 days. Severe injection site reactions did not occur in the group 4 animals or in the remaining group 5 rabbit. Gemifloxacin treatment did not affect body weights or food consumption in groups 1, 2, 3, and 5. There was no effect on body weight in group 4; food consumption for this group was not measured.

The C_{max} and AUC_{0-5h} values measured for the Group 1 (30 mg/kg/day) rabbit on day 6 were 12.4 g/ml and 19.6 gh/ml. The C_{max} and AUC_{0-5h} values measured for the Group 3 (20 mg/kg/day) rabbit on day 6 were 16.3 g/ml and 14.2 gh/ml. Plasma and AUC concentrations in these rabbits were higher than those observed after an oral dose of 30 mg/kg which was associated with reduced food consumption and body weight gain in a previous rabbit dose range finding study.

The results from the group 4 animals suggest that keeping the concentration of gemifloxacin in the infusion solution ≤ 2 mg/ml and following the infusion with a saline flush helped prevent the irritation site injection observed in the other dose groups. Concentrations of gemifloxacin ≥ 4 mg/ml in the infusion solution were associated with severe injection site reactions, even when the infusion was slowed and followed with a saline flush as in Group 5 (one rabbit in this group had an injection site reaction and the other did not).

The investigators thought it possible that intravenous infusion may be useful to achieve adequate systemic exposure in rabbits to allow them to be used for a reproduction toxicity study, provided that injection site reactions severe enough to suspend dosing could be avoided.

SB 265805: An Intravenous Infusion Maximum Tolerated Dose Study in the Female Rabbit (SB Document No. SB-265805/RSD-10110M/1; Protocol No. G98143)

Report issued 7/21/99, U.S. GLP

Vol. 1.5.024

Animals: Female nonpregnant New Zealand White rabbits, about 5 months old at receipt, 2.7-4.3 kg at the initiation of dosing, 2/dose group

Diet: _____ Rabbit Chow _____ was offered daily (180 g/day) and tap water that

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had been purified by reverse osmosis and sterilized with ultraviolet light was available *ad libitum*.

Drug Dose and Route of Administration: Gemifloxacin (Lot No. EF03-13R1P5[1]) was dissolved in 0.9% sodium chloride and administered daily as a one hour continuous infusion into the femoral vein at doses of 10, 20, 40, or 100 mg/kg (based on free drug). The concentration of gemifloxacin in the dosing solutions for each group were 1, 2, 4, and 10 mg/ml, respectively, so that a volume of 10 ml/kg was administered at each infusion. Vascular catheters were implanted one week before phase 1 of the study was initiated. Jackets were used to keep the catheter and tether in place. There were one or two occasions during the course of the study where individual rabbits received less than the planned dose due to catheter kink or occlusion or a pump system error.

Length and Conduct of Study: The rabbits received IV infusions for 15 consecutive days and were sacrificed on day 16. The study was performed in 3 phases. In the first phase, the 10 and 20 mg/kg dose groups received infusions. The data from previous phases were used to set the dose for the next. In the second phase (initiated about 4 weeks after phase 1 was completed), the 40 mg/kg group was studied and in the third phase (initiated about 1 week after phase 2 was completed), the dose chosen was 100 mg/kg.

Rabbits were checked twice daily for clinical signs. Body weights and food consumption were measured daily during the dosing period.

The investigators attempted to collect blood (into EDTA) from each on day 6 (auricular artery or marginal ear vein) 1 minute before the end of infusion, then 10 and 30 minutes, and 1, 2, 5, 10, and 24 hours after infusion. In groups 1 and 2, sampling problems occurred, so they attempted to sample on day 7 according to the same time frame. As this was also unsuccessful, the sampling process was also repeated on day 14 for these groups. The other groups had blood samples collected on day 6. Plasma samples were shipped to SKB and the levels of gemifloxacin were measured using _____ with a lower limit of quantitation of ~ ng/ml.

Results: None of the rabbits died during the course of the study. Neither body weight nor food consumption was affected at 10 or 20 mg/kg of gemifloxacin. In the 40 mg/kg group, food consumption and body weight were not affected in one animal, but the other lost 6% of its body weight and had food consumption reduced 47-86% during days 8-16. At the 100 mg/kg dose, one rabbit lost 8% of its body weight and the other 16%. These rabbits experienced a reduction of 75-98% in food consumption during days 2-16 of the study.

Dose-related infusion site reactions were observed in the rabbits. The area at the infusion site was firm in both 10 mg/kg rabbits and one 20 mg/kg animal. Thickening was observed in the other 20 mg/kg rabbit and in one at 40 mg/kg. Masses were seen at the infusion site in the other 40 mg/kg animal and in both 100 mg/kg rabbits. The pathology report stated that the masses were well-defined, firm, and pale. Severe necrotizing phlebitis/periphlebitis was associated with the masses. Fat lesions secondary to the infusion site mass were observed in one of the 100 mg/kg rabbits. The dilatation of

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the ureter of this rabbit appeared secondary to the infusion site/fat lesions (adhesion between the ureter and the mass was observed) and the kidney associated with this ureter was enlarged. Kidney changes that may have been drug-related were observed in one 40 mg/kg rabbit (enlargement and paleness of right kidney) and one 100 mg/kg rabbit (bilateral multiple pale, firm raised areas in cortex).

Cmax and AUC increased with dose. The rise was dose proportional between 10 and 20 mg/kg, but it was greater than dose proportional at 40 and 100 mg/kg, particularly the latter. Plasma levels of gemifloxacin could be quantified up to about 11 hours after infusion in the 2 lower dose groups and up to 24 hours in the 2 higher dose groups.

Mean Toxicokinetic Parameters in Rabbits Following 1 Hour Gemifloxacin Infusions (n=2)

		10 mg/kg	20 mg/kg	40 mg/kg	100 mg/kg
Cmax (g/ml)	Day 6	1.37	4.53	13.0	41.7
	Day 14	2.07	4.25	---	---
AUC _{0-t} (gh/ml)	Day 6	2.52	8.71	29.9	162
	Day 14	3.36	9.51	---	---

Body weight and food consumption in rabbits were not affected by 10 or 20 mg/kg of gemifloxacin infused daily over 1 hour for 15 days. The infusion-site lesions observed in these groups were not severe (firmness or thickening were observed). One animal at 40 mg/kg had reduced food consumption during the second week of dosing and lost weight. This rabbit also had a mass at the infusion site, while the other in this dose group did not. The 100 mg/kg dose of gemifloxacin was associated with severe injection site lesions, markedly reduced food consumption and body weight loss. One animal each in the 40 and 100 mg/kg dose groups had kidney changes that may have been gemifloxacin-related. The maximum tolerated dose in this study was 40 mg/kg.

SB 265805: Intravenous Infusion Dose Range Study for Effects on Embryo-Fetal Development in Rabbits (SB Document No. SB-265805/RSD-10110R/1; Protocol No. G99028)

Report issued 10/27/99, U.S. GLP

Vol. 1.5.024

Animals: Inseminated female New Zealand White rabbits, about 5 months old and 3.0-3.8 kg at the initiation of dosing, 6/dose group

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NDA 21,158-000/Factive (gemifloxacin)

Diet: Rabbit Chow was offered daily (180 g/day) and tap water that had been purified by reverse osmosis and sterilized with ultraviolet light was available *ad libitum*.

Drug Dose and Route of Administration: Gemifloxacin (Lot No. EF03-14R1P5) was dissolved in 0.9% sodium chloride and administered daily as a one hour continuous infusion into the vena cava at doses of 0 (vehicle control), 10, 20, or 40 mg/kg (based on free drug). The concentration of gemifloxacin in the dosing solutions for each group were 1, 2, or 4 mg/ml, respectively, so that a volume of 10 ml/kg was administered at each infusion. Catheters were implanted one week before insemination. Jackets were used to keep the catheter and tether in place and the rabbits were continuously infused with 0.9% saline for injection at 1.8 ml/h in between gemifloxacin infusions. There were several occasions during the course of the study where individual rabbits received less than the planned dose due to catheter kink or occlusion or a pump system error. Additionally, dosing had to be stopped in some of the rabbits prior to day 19 because their catheters were cut (one rabbit in the 20 mg/kg group on day 13 and two rabbits in the 40 mg/kg group on days 14 and 18).

Length and Conduct of Study: The rabbits received IV infusions from days 7-19 of gestation and were sacrificed on day 29. Gross necropsy was performed on the does and the kidneys were sliced open (left cut transversely and right cut horizontally) so that the cortex, medulla, and pelvis could be examined. Corpora lutea were counted and the gravid uterus weighed. The uterine contents were examined (including placentas, resorption and implantation sites) and fetal viability and gender were determined. Fetuses were weighed and examined for external malformations.

Rabbits were checked twice daily for clinical signs. Body weights were recorded on days 0, 3, 7-20, 24, and 29 of gestation and food consumption was measured daily during gestation.

The investigators collected blood (into EDTA) from each rabbit on day 9 of gestation (auricular artery or marginal ear vein) within 30 minutes before the initiation of the infusion, 5 minutes before the end of infusion, then 10 and 30 minutes, and 1, 2, 5, 10, and 23 hours after infusion. Plasma samples were shipped to SKB and the levels of gemifloxacin were measured using _____ with a lower limit of quantitation of _____ng/ml.

Results: None of the rabbits died during the course of the study. All of the rabbits were pregnant. Decreased fecal output related to reduced food consumption was observed in 3/6 rabbits at 40 mg/kg and 1/6 at 20 mg/kg. One control animal aborted on day 22 of gestation.

Mean body weight gain in the 40 mg/kg group was less than control (0.34 kg vs. 0.17 kg), but the difference was not statistically significant. Mean food consumption in the 20 mg/kg group was reduced during the first week of dosing (about 13-36%; with

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statistical significance observed at only the day 9-10 interval), but not during the remainder of dosing or gestation. Mean food consumption in the 40 mg/kg group was reduced during entire the dosing period (up to 38%) with statistical significance observed at several intervals.

The numbers of corpora lutea and implantation sites were similar between dose groups. Preimplantation loss and postimplantation loss were not affected by gemifloxacin treatment. No external malformations were observed in any of the fetuses. Mean fetal weight (\pm SD) was reduced in the 40 mg/kg group compared to control (47.4 ± 7.22 vs. 39.1 ± 6.51) and this reduction appeared related to gemifloxacin.

Cmax and AUC rose with dose, but the increases appeared to be slightly greater than dose proportional. Plasma levels of gemifloxacin could be quantitated up to 11 hours after the start of infusion. About half of the 40 mg/kg rabbits still had quantifiable plasma levels of gemifloxacin at the 24 hour time point.

**Mean (\pm SD) Toxicokinetic Parameters in Pregnant Rabbits
Following a 1 Hour Gemifloxacin Infusion**

Dose (mg/kg)	Cmax (g/ml)	AUC _{0-t} (gh/ml)
10	2.6 ± 0.822	3.69 ± 0.618
20	8.16 ± 0.986	12.2 ± 2.59
40	15.1 ± 3.49	28.2 ± 7.12

Reduced food consumption occurred in pregnant rabbits during the first week of dosing at 20 mg/kg and during the whole dosing period at 40 mg/kg. Body weight gain was reduced at 40 mg/kg. Decreased fetal weight was observed at 40 mg/kg.

SB 265805: Intravenous Infusion Study for Effects on Embryo-Fetal Development in Rabbits (SB Document No. SB-265805/RSD-10110S/2; SB Study No. G99029; Project No. 96593)

Report issued 10/26/99, Amendment issued 11/12/99, U.S. GLP

Vol. 1.5.025

Animals: Inseminated female New Zealand White rabbits, about 5 months old and 3.2-3.9 kg at the initiation of dosing, 22/dose group

Diet: Rabbit Chow was offered daily (180 g/day) and tap water that had been purified by reverse osmosis and sterilized with ultraviolet light was available *ad libitum*.

Drug Dose and Route of Administration: Gemifloxacin (Lot No. EF03-14R1P5) was

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dissolved in 0.9% sodium chloride and administered daily as a one hour continuous infusion into the vena cava at doses of 0 (vehicle control), 10, 20, or 40 mg/kg (based on free drug). The concentration of gemifloxacin in the dosing solutions for each group were 1, 2, or 4 mg/ml, respectively, so that a volume of 10 ml/kg was administered at each infusion. Catheters were implanted one week before insemination. Jackets were used to keep the catheter and tether in place and the rabbits were continuously infused with 0.9% saline for injection at 1.8 ml/h in between gemifloxacin infusions. Several animals (from various dose groups) had to be replaced prior to the initiation of dosing due to cut or blocked catheters. An additional animal was added to the 20 mg/kg dose group because 2 rabbits from this group had dosing suspended on day 7 or 8 of gestation due to a cut catheter. These animals were excluded from analysis. There were several occasions during the course of the study where individual rabbits received less than the planned dose due to catheter kink or occlusion or a pump system error. Additionally, dosing had to be stopped in one rabbit in the 20 mg/kg group on day 14 because its catheter was cut.

Length and Conduct of Study: The rabbits received IV infusions from days 7-19 of gestation and were sacrificed on day 29. Gross necropsy was performed on the does and the kidneys were sliced open (left cut transversely and right cut horizontally) so that the cortex, medulla, and pelvis could be examined. Corpora lutea were counted and the gravid uterus weighed. The uterine contents were examined (including placentas, resorption and implantation sites) and fetal viability and gender were determined. Fetuses were weighed and examined for external malformations. The fetal brains were examined *in situ* after a cross sectional cut was made between the parietal and frontal bones. Eyelids were removed so that anophthalmia or microphthalmia could be identified. The abdominal and thoracic viscera of all fetuses were examined using a modified Staples' dissection technique. Following evisceration, the fetuses were fixed, then clarified and stained with alizarin red S for skeletal examination.

Rabbits were checked twice daily for clinical signs. Body weights were recorded on days 0, 3, 7-20, 24, and 29 of gestation and food consumption was measured daily during gestation.

Results: One control rabbit was found dead on day 17 of gestation and another was sacrificed in moribund condition on day 18. Gross necropsy did not reveal definitive causes of death for these animals, but both had lesions at the infusion site. One rabbit from the 40 mg/kg group was sacrificed on day 17 of gestation because one of its hindpaws had become ulcerated following self-mutilation. One rabbit each in the control and 40 mg/kg groups and 2 rabbits each in the 10 and 20 mg/kg groups were not pregnant. One rabbit each in the 10 and 40 mg/kg dose groups had total resorption of their litters. Three rabbits in the control and 40 mg/kg groups and 2 in the 20 mg/kg group aborted their litters prior to cesarean section (one of the control rabbits only partially aborted its litter).

Body weight gain did not differ significantly between the treatment groups. However, reduced mean food consumption occurred during the treatment period in the 40

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mg/kg dose group. Reduced fecal output secondary to reduced food consumption was observed in 7/22 rabbits in the 40 mg/kg group on several occasions. Gross necropsy of the does revealed firmness and/or thickening at the infusion site (vena cava). The incidence of the lesions was greater in the gemifloxacin-treated groups compared to control and was likely to have been secondary to an irritating effect of the drug. The severity of the lesions tended to be dose-related.

The numbers of corpora lutea and implantation sites and preimplantation loss were similar between the treatment groups. There was no evidence of a gemifloxacin-related effect on postimplantation loss.

Mean fetal weights (\pm SD) were lower in the 40 mg/kg group than controls (46.20 ± 4.07 vs. 42.65 ± 7.12). Although statistical significance was achieved only when the female fetuses in these dose groups were compared, the effect appeared to be biologically significant for the males and for the sexes combined. The report mentioned that the mean fetal weights for the 40 mg/kg group were also lower than the historical control data for rabbit teratology studies conducted using IV infusion as the route of administration for test substances. Treatment with gemifloxacin did not increase the incidence of fetal anomalies or variations (external, visceral, or skeletal) observed in the rabbit fetuses. In fact, a greater incidence of fetuses with major malformations was observed in the control group than in the gemifloxacin groups. Types of major malformations observed in the fetuses included hydrocephaly, micrognathia, omphalocele, vertebral column abnormalities, and abnormal limb flexure.

Reduced mean food consumption in the does from the 40 mg/kg dose group during the gemifloxacin treatment period appeared to be a sign of slight maternal toxicity. The mean weight of fetuses from this dose group was less than controls. This could be related to the reduction in maternal food consumption or a sign of slight fetotoxicity. Gemifloxacin was not associated with an increase in external, visceral, or skeletal malformations in rabbit fetuses from does given up to 40 mg/kg/day via intravenous infusion during days 7-19 of gestation.

SB 265805: Oral Study for Effects on Pre- and Postnatal Development in Rats (SB Document No. SB-265805/RSD-10110P/2; Protocol No. G98130)

E.A. Zahalka, A. Mirabella (SmithKline Beecham, King of Prussia, PA)

Report issued 7/23/99, amended 10/5/99, U.K., U.S., and OECD GLP

Vol. 1.5.026

Animals: Timed-mated female Sprague-Dawley rats, approximately 10 weeks old and 200 g at time of cohabitation, 24 per group for reproduction study, 5 satellite animals per group for toxicokinetics

NDA 21,158-000/Factive (gemifloxacin)

Diet: Certified Rodent Chow _____ and filtered tap water were available *ad libitum*.

Drug Dose and Route of Administration: Gemifloxacin (Lot No. 03-13R1P5) was dissolved in 0.9% sodium chloride and administered via oral gavage (20 ml/kg) at doses of 0 (vehicle control), 30, 270, or 750 mg/kg/day (based on the mesylate salt) equivalent to 24, 216, and 600 mg/kg/day for the gemifloxacin free base.

Length and Conduct of Study: Dams were dosed orally from day 6 of gestation through day 20 of lactation. Dams were sacrificed on day 21 of lactation.

For toxicokinetic evaluation, satellite rats were dosed with drug on days 6-9 of gestation. Blood samples were collected prior to dosing on day 9, then 1, 2, 4, 6, 8, 12, and 24 hours after gemifloxacin was administered. Serum was assayed for gemifloxacin using _____ (lower limit of detection was _____ ng/ml).

Rats in the reproduction study were checked daily for viability, clinical signs of drug toxicity, and maternal behavior. The body weight of the F0 dams was measured daily from day 6 of gestation to day 21 of lactation. Food consumption for the F0 dams was measured at these intervals: days 6-10, 10-14, 14-19, 19-21 of gestation and days 1-4, 4-8, 8-12, and 12-14 of lactation. Any dam which had not delivered by day 24 of presumed gestation was sacrificed and its uterus (including any uterine contents) examined. Dams with no live pups were also sacrificed.

As soon as practical after parturition, pups were counted, weighed, sexed, and examined externally. Dead and malformed offspring were removed, then examined for visceral abnormalities. The lungs of dead pups were examined to see if the pup had been born alive or if it was stillborn. The pups' body weight was measured on days 0, 1, 4, 7, 14, and 21 of lactation. On day 21 of lactation, 3 pups per sex were selected from each litter for further testing (the first pair for reproductive testing; the second for auditory and tactile startle reflexes and spontaneous locomotor activity; the third for auditory and tactile startle reflexes and passive avoidance learning/retention tests). Body weights of these pups were measured on days 28, 35, 42, 56, 70, 84, and 98 post partum. The females were examined beginning on day 27 post partum to determine the day of vaginal opening and the males were examined beginning on day 40 post partum to determine the day of balano-preputial skin fold separation. Two pups per sex per litter were then randomly chosen for skeletal examination following sacrifice, evisceration, and processing/staining with alizarin red S. ...

For the F1 reproduction tests, estrous cycles of females were evaluated by daily vaginal lavage from days 64-73 post partum. Beginning on day 74 post partum, males and females from different litters (but the same dose group) were cohabitated 1:1 for up to 14 days, until sperm was present in vaginal lavage. Females that did not show signs of mating were sacrificed 21 days after the end of cohabitation and their uteri examined for implantation sites. Mated females were weighed on day 0 of gestation, then weekly until delivery. These rats were allowed to deliver their litters. The pups were weighed as soon after birth as possible, then counted, sexed, and examined externally. They were weighed on days 1 and 7 of lactation as well. F1 dams and their litters were sacrificed on day 7 of

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lactation.

The second set of F1 pups had their startle reflexes to auditory (animals placed in lighted box with noise generator and movement monitor) and tactile stimuli (animals placed in box and exposed to puff of air) tested once between days 27-30 post partum. Spontaneous locomotor activity (animal placed in cage with movement monitor) was measured for about 23 hours once during days 54-61 post partum (an amphetamine challenge, 0.5 mg/kg, was injected subcutaneously about 22 hours after the initiation of measurement).

The third set of F1 pups was evaluated in the passive avoidance test for learning and retention (shuttle box with lighted side and dark side- electric shock given when rat entered the dark side) during days 40-50 post partum. Retention was tested one day after the animals were placed in the shuttle box for the learning trial. The startle response to auditory and tactile stimuli was tested once (as above) between days 70-77 post partum.

Results: None of the F0 dams died during the study. All were pregnant. Salivation was observed prior to dosing in 11/24 rats in the 270 mg/kg group and 24/24 rats in the 750 mg/kg group. It was also observed after dosing in 11/24 rats in the 750 mg/kg group.

One rat in the 750 mg/kg group had not given birth by day 24 post coitus. Necropsy revealed 8 implantation sites and one placenta in the uterus. All of the pups in one control litter died on day 1 of lactation and all pups in one 750 mg/kg litter died on day 0.

Body weight gain in the 750 mg/kg group was lower than control during days 6-10 (about 36% lower gain) and 14-19 (about 32% lower gain) of gestation. On days 19-21 of gestation (close to delivery), the mean body weight of this high dose group was significantly (7-8%) less than control. The dams in the 750 mg/kg group also ate significantly less food than controls during days 6-19 of gestation (up to 27% less). During the lactation period, despite continued dosing with gemifloxacin, the dams in the 750 mg/kg group consumed a comparable amount of food as controls and the mean body weights did not differ between the 2 groups by day 8 of lactation.

The mean gestational length in the 750 mg/kg group was slightly greater than concurrent control (21.1 vs. 21.6 days), but it was within the historical control range according to the investigators. The time it took to deliver the litters (among births that were observed) did not differ between treatment groups and dystocia was not observed. Thus, it did not appear that gemifloxacin at doses up to 750 mg/kg caused prolonged gestation or adverse effects on parturition.

The mean number of implantation sites was similar across dose groups. The mean number of live pups per litter did not differ between the control or gemifloxacin groups, nor did neonatal survival or survival during the lactation period (mean percent viability). At birth, mean pup body weight for both males and females was significantly lower than control in the 750 mg/kg dose group, but at the end of the lactation period, the mean pup body weight at 750 mg/kg was not significantly different from control, though it was still lower. Skeletal examination of the selected pups from each treatment group demonstrated no gemifloxacin-related effects on ossification. These observations suggest that the growth retardation and delayed ossification observed in previous rat reproduction

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toxicity studies is not permanent, but reversible.

An increased incidence of eye defects (anophthalmia or microphthalmia- 12 pups from 3 litters) and dome shaped head (likely hydrocephaly- 3 pups from 3 litters) was observed in the 750 mg/kg pups. Overall, 14 pups from 5 litters had eye defects and/or dome shaped head. These malformations were not observed in the control or lower dose gemifloxacin groups. None of the other malformations observed in a few pups (e.g., edema, cardiac or lung malformations, diaphragmatic hernia) appeared related to gemifloxacin.

Gemifloxacin treatment did not affect the attainment of the developmental landmarks balanopreputial separation in males or vaginal opening in females. The responses of the pups to auditory or tactile startle stimuli did not differ in a biologically significant manner between control or gemifloxacin groups at either testing time point (age 27-29 days or 70-77 days). Gemifloxacin treatment did not appear to alter the response of the pups to a passive avoidance test (learning or retention trial), nor was it associated with changes in motor activity (including amphetamine challenge).

The reproductive performance of the F1 generation was not affected by gemifloxacin at doses up to 750 mg/kg. The estrus cycle of the female rats and the time to mating was not significantly altered and mating and pregnancy incidences were comparable between treatment groups. Gestation length, average pup delivery time, and the mean numbers of implantation sites and live pups per litter were similar between treatment groups. F2 pup body weights and neonatal and perinatal survival did not differ based upon parental gemifloxacin treatment. External examination of the F2 pups did not reveal drug-related abnormalities.

The serum concentrations of gemifloxacin in pregnant rats (n = 5 per group) following oral administration of the drug increased with dose. In the 30 mg/kg group, gemifloxacin could be detected in serum up to at least 12 hours after dosing and in the two higher dose groups, the drug could be detected up to 24 hours after administration. The serum concentrations of gemifloxacin in the current study were higher than those observed in a previous rat fertility and embryonic development study using the same doses of drug.

**Mean (\pm SD) Toxicokinetic Parameters in Pregnant Rats
After Oral Administration of Gemifloxacin**

Dose (mg/kg)	Tmax (h) (w/-range)	Cmax (g/ml)	AUC_{0-t} (gh/ml)
30	1.08 (1.07-1.15)	0.465 \pm 0.085	2.32 \pm 0.45
270	1.12 (1.08-2.10)	3.91 \pm 1.63	22.1 \pm 5.2
750	1.10 (1.07-1.13)	10.2 \pm 2.5	73.8 \pm 15.9

In the 750 mg/kg gemifloxacin dose group, F0 dams consumed significantly less

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food than controls and gained less weight during gestation. Despite continued gemifloxacin treatment, these rats consumed a similar amount of food as controls during the lactation period and mean body weight did not differ significantly between the 2 groups by day 8 of lactation. Pup birth weights in the 750 mg/kg group were significantly lower than control, but by day 21 of lactation, body weights no longer differed significantly and skeletal ossification did not differ between F1 rats in the control and high dose groups. These observations suggest that the fetal growth retardation observed in this study and in the previous rat fertility and embryonic development study is reversible. Eye malformations and dome shaped head were observed in some pups from the 750 mg/kg group in the current study (overall, 14 pups from 5 litters). These malformations were not seen in the previous rat fertility and early embryonic development study, but it should be noted that the serum concentration in the current study are higher than the plasma concentrations seen in the former study. The reason for the difference is not clear- the same strain of rat was used for both studies although they were from different suppliers. The only other difference was that a larger dose volume was used in the current study. Attainment of developmental landmarks and behavioral test results (startle response, passive avoidance learning/retention, spontaneous activity) in the F1 pups were not affected by maternal gemifloxacin treatment from day 6 of pregnancy throughout the lactation period at doses up to 750 mg/kg. Reproductive function in the F1 generation was also unaffected at the same doses.

Genetic Toxicology Studies:

Induction of Chromosome Aberrations in Cultured Chinese Hamster Ovary (CHO) Cells in the Presence of Ultraviolet Light (SB Document No. SB-265805/RSD-100X0S/3; Protocol No. G98640; Study No. 802/487)

Report dated 6/30/99; Amended 10/25/99; — and —GLP

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Results: Chromosome aberrations were observed in CHO cells exposed to gemifloxacin in the absence of UV and an increase in aberrations was not observed in the UV-exposed cultures. Chromosome aberrations were also observed in the absence of UV in the ciprofloxacin-treated cells, but UV exposure caused an increase in ciprofloxacin clastogenicity. Lomefloxacin did not produce chromosome aberrations in the absence of UV, but clastogenic activity was observed when lomefloxacin and UV exposure were combined. Positive and solvent controls gave expected responses.

**Fluoroquinolone-Induced Chromosome Aberrations in CHO Cells
in the Presence and Absence of UV Exposure**

Treatment (g/ml)	% Cells with Aberrations (Excluding Gaps)	
	No UV	+ UV
Gemifloxacin		
60.03	40.5*	40.5*
122.5	79*	68*
250	59*	67*
Saline	3.5	4
Ciprofloxacin		
245	4	10**
350	11.5*	22*

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