

**CENTER FOR DRUG EVALUATION AND RESEARCH**

**APPLICATION NUMBER: 20759/20760**

**PHARMACOLOGY REVIEW(S)**

866.5  
520

DEC 15 1997

NDA's 20,759-000 and 20,760-000/Trovan® Tablets and IV

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

**NDA #s:** 20,759-000 (trovafloxacin mesylate tablets) and 20,760-000 (alatrofloxacin mesylate injection)

**SPONSOR:** Pfizer Inc.  
Eastern Point Road  
Groton, CT 06340

ADP 12/30/96  
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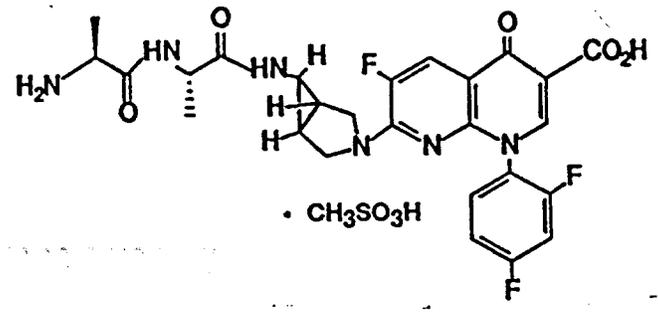
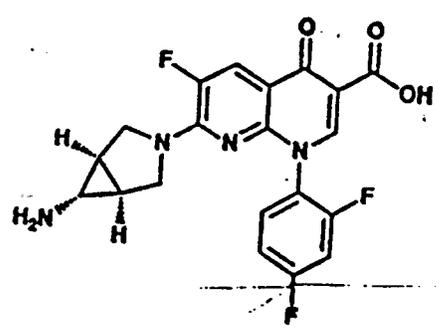
**AUTHORIZED REPRESENTATIVE:** Ronald I. Trust, Ph.D., M.B.A.  
(860) 441-6991

**DRUG NAMES:** Trovan® Tablets: trovafloxacin mesylate; CP-99,219-27; (1 $\alpha$ ,5 $\alpha$ ,6 $\alpha$ )-7-(6-amino-3-azabicyclo[3.1.0]hex-3-yl)-1-(2,4-difluorophenyl)-6-fluoro-1,4-dihydro-4-oxo-1,8-naphthyridine-3-carboxylic acid monomethanesulfonate

Trovan® I.V.: alatrofloxacin mesylate; CP-116,517-27; (1 $\alpha$ ,5 $\alpha$ ,6 $\alpha$ )-L-alanyl-N-[3-[6-carboxy-8-(2,4-difluorophenyl)-3-fluoro-5,8-dihydro-5-oxo-1,8-naphthyridin-2-yl]-3-azabicyclo[3.1.0]hex-6-yl]-L-alaninamide monomethanesulfonate

**CATEGORY:** Fluoronaphthyridone anti-infective (structurally similar to fluoroquinolones and also an inhibitor of DNA gyrase)

**STRUCTURAL FORMULAS:**



Alatrofloxacin is the bis L-alanine prodrug of trovafloxacin.

**RELATED SUBMISSIONS:** IND

**NUMBER OF VOLUMES:** 203 total (18 for Pharm/Tox)

**DATE CDER RECEIVED:** 12/30/96

**DATE ASSIGNED:** 1/7/97

**DATE REVIEW STARTED:** 1/8/97

**DATE 1<sup>ST</sup> DRAFT COMPLETED:** 12/11/97

**DATE REVIEW ACCEPTED BY TEAM LEADER:** *December 15, 1997*

**REVIEW OBJECTIVES:** To ascertain whether the nonclinical studies submitted by the sponsor adequately demonstrate the potential toxicities of trovafloxacin and alatrofloxacin and to determine if these drug products meet safety standards allowing them to be approved for marketing.

**PROPOSED DOSAGE FORM AND ROUTE OF ADMINISTRATION:**

Trovafloxacin will be available as 100 and 200 mg tablets (based on weight of trovafloxacin free base) to be administered orally. Each tablet contains:

	100 mg tablet	200 mg tablet
Trovafloxacin Mesylate <sup>a</sup>		
Microcrystalline Cellulose <sup>b</sup>		
Magnesium Stearate		
coating		

<sup>a</sup>Weight of drug substance will be adjusted based on actual potency of lot

<sup>b</sup>Weight of microcrystalline cellulose will be adjusted depending on amount of trovafloxacin mesylate used in the batch in order to maintain constant tablet weights  
tablet.

contains hydroxypropyl methylcellulose, hydroxypropyl cellulose, titanium dioxide, polyethylene glycol, and FD&C blue #2 aluminum lake.

Alatrofloxacin will be available as aqueous 5 mg/ml concentrates in vials containing 100, 200, or 300 mg (all based on weight of trovafloxacin free base equivalent) to be diluted prior to intravenous administration.

The solution will be adjusted to pH of approximately 3.75 using HCl and NaOH as needed. For example, a 100 mg vial will contain 157.232 mg of alatrofloxacin mesylate in 20.06 ml of water.

**REQUESTED CLINICAL INDICATIONS:**

<b>Indication</b>	<b>Daily Dose/Route</b>	<b>Treatment Duration</b>
<b>Adults</b>		
<b>Nosocomial Pneumonia</b>	300 mg I.V followed by 200 mg oral	10-14 days
<b>Community Acquired Pneumonia</b>	200 mg oral or 200 mg I.V. followed by 200 mg oral	7-14 days
<b>Acute Bacterial Exacerbation of Chronic Bronchitis</b>	100 mg oral	7-10 days
<b>Acute Sinusitis</b>	200 mg oral	10 days
<b>Complicated Intra- Abdominal Infections, including post-surgical infections</b>	300 mg I.V. followed by 200 mg oral	7-14 days
<b>Gynecologic and Pelvic Infections, Complicated, including post-surgical infections</b>	300 mg I.V. followed by 200 mg oral	7-14 days
<b>Surgical Prophylaxis- Elective Colorectal Surgery</b>	200 mg I.V.	Single dose within 4 hours before surgery
<b>Surgical Prophylaxis- Elective Abdominal and Vaginal Hysterectomy</b>	200 mg oral	Single dose within 4 hours before surgery
<b>Skin and Skin Structure Infections, Uncomplicated</b>	100 mg oral	7-10 days
<b>Skin and Skin Structure Infections, Complicated, including diabetic foot infections</b>	200 mg oral or 200 mg I.V. followed by 200 mg oral	10-14 days
<b>Uncomplicated Urinary Tract Infections, including cystitis</b>	100 mg oral	3 days

Bacterial Prostatitis	200 mg oral	28 days
Acute, Uncomplicated Gonorrhea	100 mg oral	Single Dose
Non-Gonococcal Urethritis and Cervicitis	200 mg oral	5 days
Pelvic Inflammatory Disease	200 mg oral or 200 mg I.V. followed by 200 mg oral	14 days
Children		

**INDEX OF NONCLINICAL STUDIES SUBMITTED TO THIS NDA**  
**(and location of review):**

**Pharmacology Studies:**

**General Pharmacology of CP-99,219-27** Summary reports of studies investigating the general pharmacologic parameters of trovafloxacin are included here. *In vivo* experiments explored the effects of trovafloxacin on CNS activity in mice, canine cardiovascular system, renal excretion in conscious rats, blood gas measurements in rats, gastrointestinal transit time in rats, and gastric acid secretion in pylorus-ligated rats. *In vitro* experiments explored trovafloxacin-induced effects on ligand binding, isolated guinea pig aorta, ileum, and atria, and isolated rat uterus.

Reviewed below

**General Pharmacology of CP-116,517** Summary reports of a study investigating the effects of alatrofloxacin on the cardiovascular system of conscious primates and an *in vitro* study of alatrofloxacin effects on receptor-specific ligand binding in bovine brain.

Reviewed below

**Interaction of Trovafloxacin and Alatrofloxacin with a Non-Steroidal Anti-Inflammatory Drug in Mice** Summary Study Report

Reviewed below

**Toxicology Studies:**

**Single Dose Toxicity Studies:**

**Trovafloxacin:**

**Single Dose Oral and Intraperitoneal Toxicity Studies in Mice and Rats (91-783-14)**

**Alatrofloxacin:**

**Single Dose Intravenous Toxicity Studies in Mice and Rats (92-851-10)**

**Multiple Dose Acute Toxicity Studies:**

**Trovafloxacin:**

**14 Day Oral Gavage Exploratory Toleration Study in Sprague-Dawley Rats at 0, 50, 100, or 200 mg/kg/day (91-783-06)**

**14 Day Oral Exploratory Toleration Study in Beagle Dogs at 0, 12.5, 25, and 50 mg/kg/day (91-783-07)**

**Alatrofloxacin:**

**2 Week IV Exploratory Toleration Study in Sprague-Dawley Rats (92-851-07)**

**Ten Day Intravenous Infusion Exploratory Toleration Study in Beagle Dogs (92-851-08)**

**Subchronic/Chronic Toxicity Studies:**

**Trovafloxacin:**

**One Month Oral Toxicity Study in Beagle Dogs (91-783-09)**

**One Month Oral Toxicity Study in Sprague-Dawley Rats (91-738-10)**

**6 Month Gavage Study in Beagle Dogs (93-783-16)**

**6 Month Oral Gavage Study in Sprague-Dawley Rats (93-783-17)**

**Alatrofloxacin:**

**1 Month Intravenous Toxicity Study in Sprague-Dawley Rats at 6.5, 20, and 50 mg/kg (92-851-04)**

**1 Month Intravenous Toxicity Study in Beagle Dogs (92-851-05)**

**Special Toxicity Studies:**

**Trovafloxacin:**

**Antigenicity Study in Guinea Pigs (94-01-81)  
reviewed below**

**Six Month Oral Gavage Reversibility Study in Beagle Dogs (94-783-23)**

**An Acute Phototoxicity Study in Balb/c Mice Dosed Orally (95-783-27)**

**8-Week Oral (Gavage) Subchronic Study of CP-99,219-27 in Hairless Mice, With or Without Added Simulated Sunlight (C-516-001)  
reviewed below**

**12-Month Oral (Gavage) Study to Determine the Influence of CP-99,219-27 on Photocarcinogenesis in Hairless Mice (C-516-002)  
Protocol Only- Study has been completed and will be reviewed when Final Report is submitted**

**Neonatal Study in Sprague-Dawley Rats (94-783-22)**

**Exploratory Neonatal Study in Sprague-Dawley Rats (95-783-28)**

**Exploratory Neonatal Study in Sprague-Dawley Rats (95-783-29)**

APPROVED FOR RELEASE  
BY [illegible]

**Exploratory Neonatal Study in Sprague-Dawley Rats (95-783-30)**  
reviewed below

**A Two-Week Range Finding Study of CP-99,219-27 in Neonatal Beagle Dogs  
94461)**  
reviewed below

**A Four-Week Oral Toxicity Study of CP-99,219 in Neonatal Beagle Dogs 94485)**

**CP-395,013, CP-455,276, and CP-425,111-27 [Trovafloracin Mesylate (CP-99,219-27)  
Impurities] Single Dose Oral Toxicity Study in Rats (96-1418-01)**

**Alatrofloracin:**

**Antigenicity Study in Guinea Pigs (94-27-81)**

**A Single Dose Oral Toxicity Study in Rats and a Single Dose Dermal Toxicity Study in  
Rabbits and a Single Dose Ocular Irritation Study in Rabbits (95-851-14)**  
reviewed below

**Reproductive Toxicity Studies:**

**Trovafloracin:**

**Oral Fertility and Early Embryonic Development Study (Study I) in Sprague-Dawley  
Rats (93-01-41)**

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**Oral Maternal Toxicity Study in Sprague-Dawley Rats (93-01-51)**

**CP-99,219 Oral Teratology Study (Study III) in Sprague-Dawley Rats (93-01-52)**  
reviewed below

**CP-99,219 Oral Maternal Toxicity Study in Japanese White Rabbits (93-01-71)**

**CP-99,219 Oral Teratology Study (Study III) in Japanese White Rabbits (93-01-72/93-01-  
91)**

**Reproductive Study II: Prenatal and Postnatal Development in Sprague-Dawley Rats (94-783-18)**

reviewed below

**Alatrofloxacin:**

**Reproductive Study I: Fertility and Early Embryonic Development Intravenous Study in Sprague-Dawley Rats (95-851-16)**

**Maternal Range Finding Study in Sprague-Dawley Rats (95-27-52)**

**Teratology Study (Reproductive Study III) in Sprague-Dawley Rats (96-27-53)**

**Prenatal and Postnatal Development Study (Reproductive Study II) in Sprague-Dawley Rats (95-27-61)**

**Maternal Range Finding Study in Japanese White Rabbits (95-27-71)**

**Teratology Study (Reproductive Study III) in Japanese White Rabbits (96-27-72)**

**Mutagenicity Studies:**

**Genetic Toxicology Report CP-99,219-27 (90-783-02/90-783-03)**

**Genetic Toxicology Report CP-116,517-27 (91-851-02)**

**Pharmacokinetic Studies:**

all reviewed below

**Absorption:**

**Pharmacokinetics of Trovafloxacin in Sprague-Dawley Rats Following Single Intravenous Administration of Alatrofloxacin or Trovafloxacin and Single Oral Administration of Trovafloxacin (93-099219-4)**

**Pharmacokinetics of Trovafloxacin in Beagle Dogs Following Single Intravenous Administration of Alatrofloxacin or Trovafloxacin and Single Oral Administration of Trovafloxacin** (93-099219-1)

**Pharmacokinetics of Trovafloxacin in Cynomolgus Monkeys Following Single Intravenous Administration of Alatrofloxacin or Trovafloxacin and Single Oral Administration of Trovafloxacin** (93-099219-2)

**Pharmacokinetics of Trovafloxacin in New Zealand White Rabbits Following Single Intravenous or Oral Administration of Trovafloxacin** (96-099219-4)

**Protein Binding of Trovafloxacin:**

**Serum Protein Binding of Trovafloxacin in Rats, Rabbits, Dogs, Monkeys and Humans** (92-099219-5)

**Tissue Distribution:**

**Trovafloxacin:**

**Blood/Plasma Ratios of Trovafloxacin in Rats, Dogs, Monkeys and Humans** (93-099219-3)

**Distribution of [<sup>14</sup>C] Trovafloxacin into Gastric Tissue of Swiss-Webster Mice Following Intravenous Administration** (94-099219-3)

**Tissue Distribution of Trovafloxacin in Beagle Dogs Following Oral Administration of Multiple Doses** (93-099219-5)

**Whole-Body Autoradioluminography of Female and Male Long-Evans Rats After Single Intravenous Administration of [<sup>14</sup>C] Trovafloxacin** (94-099219-1)

**Whole-Body Autoradioluminography of Female and Male Long-Evans Rats After Single Oral Administration of [<sup>14</sup>C] Trovafloxacin** (95-099219-1)

**Alatrofloxacin:**

**Whole-Body Autoradioluminography of Female and Male Long-Evans Rats After Single Intravenous Administration of [<sup>14</sup>C] Alatrofloxacin** (96-116517-2)

**Metabolism and Excretion:**

**Trovafloxacin:**

**Identification of Trovafloxacin Metabolites in Human Bile Following Oral Administration of Trovafloxacin (96-099219-2)**

**Excretion and Metabolism of Trovafloxacin in Man (96-099219-1)**

**In Vitro Hydrolysis of CP-116,517 to CP-99,219 in Neonatal and Adult Rat, Guinea Pig, Dog, Monkey, and Human Serum (93-116517-1)**

**In Vitro Metabolism of [<sup>14</sup>C] Trovafloxacin by Rat and Human Liver Slices (94-099219-4)**

**Excretion and Metabolism of Trovafloxacin in Sprague-Dawley Rats (94-099219-2)**

**Excretion and Metabolism of Trovafloxacin in Beagle Dogs (94-099219-2)**

**Alatrofloxacin:**

**Metabolism and Excretion of Alatrofloxacin in Sprague-Dawley Rats (96-116517-1)**

**REVIEWS OF NONCLINICAL STUDIES:****PHARMACOLOGY STUDIES:****General Pharmacology of CP-99,219-27 (report summarizes several studies)**

A.A. Fossa (Department of General Pharmacology, Pfizer Central Research, Groton, CT)

Report dated 2/20/91

Vol.17, pp. 57-97

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**Results from *In Vivo* Experiments:** There were no differences in behavior or motor skills between vehicle-treated mice and those treated with up to 32 mg/kg of trovafloxacin. Doses of trovafloxacin between 100-1000 mg/kg were slightly sedating in undisturbed mice (ptosis, reduced locomotor activity, postural changes) and respiration appeared slower at 30-60 minutes after dosing. At 320 mg/kg, 2/3 mice were not able to right themselves on an inverted screen. Gait disturbances, reduced muscle tone, blanched skin and suppressed corneal reflex were observed at 1000 mg/kg, but no mortality occurred within a 24 hour observation period. For the comparator, ciprofloxacin, reduced locomotor activity was observed at 1000 mg/kg, but no other changes were seen at this, or lower doses.

Conscious fasted mongrel dogs were given oral 20 or 40 mg/kg doses of trovafloxacin. Electrocardiogram, heart rate and mean arterial blood pressure were measured prior to dosing and at various time points starting at the beginning of infusion until 240 minutes after infusion. Blood samples were drawn so that plasma concentrations of trovafloxacin could be determined. Emesis was observed in both dogs receiving 40 mg/kg of drug, and the investigators believed that this made them unsuitable for cardiac evaluation although they noted that "no remarkable cardiovascular effects were seen during periods when emesis was not occurring". "Licking of jowls" (said to be a sign of "abdominal discomfort") was seen in the dogs from the 20 mg/kg group, but only one female experienced emesis. This dog was not included in the data analysis of the remaining 5 animals (3 males, 2 females). Increases in heart rate (2-6 beats per minute) and cardiac contractility (manifested by decreased Q-A interval of approximately 6-9 msec, or about 8%) were observed up to 210 minutes after dosing without a concomitant increase in mean arterial pressure. These changes from the baseline values, although modest, were significantly ( $p \leq 0.05$ ) different from current, as well as historical controls. The  $C_{max}$  for serum trovafloxacin in this study averaged  $1.85 \pm 0.7 \mu\text{g/ml}$  and occurred 2 hours after dosing. The investigators believed that the true  $C_{max}$  is likely between 1 and 2 hours as the serum concentration of trovafloxacin 1 hour after dosing was  $1.63 \pm 0.89$ . It should be noted that these plasma concentrations are likely to be below the projected human therapeutic concentration of about  $3 \mu\text{g/ml}$ .

When administered orally to fasted male Sprague-Dawley rats, trovafloxacin (10 or 20 mg/kg) did not have any effect on renal function as measured by volume of urine excreted or urine osmolarity. Additionally, urinary excretion of sodium, chloride, and potassium was not significantly different in control or trovafloxacin-treated rats.

Trovafloxacin (oral dose of 20 mg/kg) did not appear to affect respiratory function in

fed male rats (species not specified). Blood gas measurements ( $PO_2$ ,  $PCO_2$ ) and pH of arterial blood drawn at 15 minute intervals up to 75 minutes after dosing did not change significantly from baseline levels in either trovafloxacin- or vehicle-treated rats.

The rate of gastric emptying was significantly ( $p < 0.05$ ) decreased in fasted male Sprague-Dawley rats by trovafloxacin at doses of 10 mg/kg (37%) and 20 mg/kg (54%) compared to vehicle. The rate of gastric emptying was measured using a solution of sodium chromate (with  $^{51}Cr$ ) given to the animals via oral gavage 30 minutes after drug or vehicle administration. The distribution of radioactivity was assessed throughout the various segments of the GI tract. Doses of ciprofloxacin (5, 10, or 20 mg/kg) also reduced gastric emptying, but statistical significance was only reached at the highest dose which caused a 19% reduction.

Trovafloxacin (5, 20, and 40 mg/kg) did not affect gastric acid secretion in fasted male Sprague-Dawley rats. The positive control, 10 mg/kg of cimetidine, inhibited gastric acid secretion by 52%.

**Results from *In Vitro* Experiments:** The ability of trovafloxacin to compete with radiolabeled ligands for receptors in bovine brain was assessed. Concentrations of trovafloxacin up to 10  $\mu M$  did not inhibit ligand binding to the following receptors:  $\alpha_1$ ,  $\alpha_2$ ,  $\beta$ , dopamine  $D_2$ , Adenosine $_1$ , 5HT $_{1A}$ , 5HT $_2$ , Histamine  $H_1$ , Muscarinic,  $\mu$ -Opiate, or GABA. Additionally, 20  $\mu M$  trovafloxacin did not inhibit muscimol binding to the GABA receptor. Data obtained using ciprofloxacin were comparable.

Concentrations of trovafloxacin up to 10  $\mu M$  did not alter norepinephrine-induced contractions of isolated guinea pig aorta.

Preincubation of isolated guinea pig ileum with 10  $\mu M$  trovafloxacin for 30 minutes inhibited histamine (1  $\mu M$ ) induced contraction by about 52%. Concentrations of trovafloxacin  $\leq 1 \mu M$  or ciprofloxacin up to 10  $\mu M$  did not have this effect.

The rate of spontaneous beating of isolated guinea pig atrium can be increased by addition of histamine. Trovafloxacin at concentrations up to 10  $\mu M$  did not alter histamine-induced chronotropic activity in isolated guinea pig atrium.

Trovafloxacin at concentrations up to 10  $\mu M$  did not alter oxytocin-induced contractions in isolated rat uterus.

#### **General Pharmacology of CP-116,517 (report summarizes several studies)**

D.R. Knight (Department of General Pharmacology, Pfizer Central Research, Groton, CT)

Report dated 10/31/91

Vol. 17, pp. 98-111

**Results:** Conscious cynomolgus monkeys (*M. fascicularis*) were given 20 mg/kg of alatrofloxacin intravenously over a 30 minute period at a dose rate of 0.33 ml/min. Electrocardiogram, heart rate and mean arterial blood pressure were measured prior to dosing and at various time points starting at the beginning of infusion until 240 minutes after infusion. Blood samples were drawn so that plasma concentrations of alatrofloxacin and trovafloxacin could be determined. Emesis was not observed in any of the 5 subjects (3 males, 2 females),

although signs of nausea (salivation, excessive licking and mastication) were seen in one female 15-30 minutes after the start of the infusion. This dose caused emesis in 2/2 primates during a previous study when administered as an IV bolus. All of the animals showed reduced activity from the beginning of the infusion up to 90 minutes after the start. Decreases in mean arterial blood pressure (15-120 minutes after start of infusion) and heart rate (45 and 120 minutes after infusion) were observed in the alatrofloxacin-treated monkeys compared to control, but these changes were not significantly different from the baseline values. No changes in the Q-A intervals or electrocardiograms were seen. Alatrofloxacin blood levels were only detectable at the very end of the infusion ( $2.97 \pm 0.28 \mu\text{g/ml}$ ). At the end of infusion, trovafloxacin serum concentrations averaged  $17.7 \pm 2.4 \mu\text{g/ml}$ .

The ability of alatrofloxacin to compete with radiolabeled ligands for receptors in bovine brain was assessed *in vitro*. Concentrations of up to  $10 \mu\text{M}$  alatrofloxacin did not inhibit ligand binding to the following receptors:  $\alpha_1$ ,  $\alpha_2$ ,  $\beta$ , dopamine  $D_2$ , Adenosine $_1$ , 5HT $_{1A}$ , 5HT $_2$ , Histamine  $H_1$ , Muscarinic,  $\mu$ -Opiate, or GABA.

#### **Interaction of Trovafloxacin and Alatrofloxacin with a Non-Steroidal Anti-Inflammatory Drug in Mice (summary report of study)**

K. Yamanaka and F. Sawamura (Pharmacology Research Laboratory, Pfizer Pharmaceuticals, Inc., Aichi, Japan)

Not dated

Vol. 17, pp. 112-117

**Results:** Male mice (5 weeks old, , 5-7 animals per treatment group) were fasted for 14 hours, then dosed orally with one of the following quinolones in 0.3% carboxymethyl cellulose:

1. Trovafloxacin (100, 200, 500, 1000, 1500, or 2000 mg/kg)
2. Enoxacin (10, 25, 50, or 100 mg/kg)
3. Norfloxacin (50, 100, or 200 mg/kg)
4. Ciprofloxacin (100, 200, or 500 mg/kg)
5. Ofloxacin (1500 mg/kg)
6. Sparfloxacin (1500 mg/kg)
7. Tosufloxacin (1500 mg/kg)

After quinolone dosing, mice were given 100 mg/kg of biphenylacetic acid (BPAA, an active metabolite of fenbufen). Convulsions were not observed in mice treated with BPAA and vehicle (carboxymethyl cellulose). Convulsions were not observed in animals dosed with up to 500 mg/kg of trovafloxacin and BPAA, although writhing and piloerection were observed in 2/5 mice in the 500 mg/kg group. Doses of trovafloxacin  $\geq 1000$  mg/kg and BPAA were associated with convulsions and death in 1/6 or 2/6 animals. Convulsions, usually followed by death, were observed in mice dosed with BPAA and enoxacin  $\geq 50$  mg/kg (3/5 at 50 mg/kg and 5/5 at 100 mg/kg), 200 mg/kg norfloxacin (3/5), 500 mg/kg ciprofloxacin

(5/5), and 1500 mg/kg ofloxacin (2/5). Convulsions were not observed in the mice dosed with sparfloxacin or tosufloxacin followed by BPAA. Convulsions were not observed in the absence of BPAA when mice were orally dosed with up to 500 mg/kg of trovafloxacin, 100 mg/kg of enoxacin, or 200 mg/kg of norfloxacin. Sedation, ataxia, and trembling were observed at doses of trovafloxacin  $\geq$  1000 mg/kg, but convulsions were observed in only 1/6 mice at 1500 mg/kg.

Groups of 10 fasted mice (as above) were dosed intravenously with alatrofloxacin (5, 25, 50, or 75 mg/kg) or norfloxacin (10 or 25 mg/kg) in saline 1 hour after oral administration of 100 mg/kg BPAA. Another group was given norfloxacin without BPAA. Convulsions were not observed in any of the alatrofloxacin/BPAA-treated mice, although sedation and ataxia were noted. Convulsions were seen in 2/10 mice treated with BPAA and 10 mg/kg norfloxacin and 7/10 treated with BPAA and 25 mg/kg norfloxacin. In the absence of BPAA, these doses of norfloxacin did not cause convulsions in mice.

The combination of trovafloxacin with 100 mg/kg of BPAA did not enhance the convulsant activity of this quinolone in mice. For oral dosing, the rank order of potency for the quinolones tested in the presence of BPAA was enoxacin > norfloxacin > ciprofloxacin > ofloxacin > trovafloxacin > sparfloxacin, tosufloxacin. When both drugs were administered intravenously after dosing with BPAA, alatrofloxacin at doses up to 75 mg/kg did not cause convulsions in mice, but doses of norfloxacin  $\geq$  10 mg/kg did.

## **TOXICOLOGY STUDIES:**

### **Special Toxicology:**

#### **Antigenicity Study in Guinea Pigs (Study No. 94-01-81)**

H. Yamada (New Product Development Center, Pfizer Pharmaceuticals Inc., Japan)

Report dated 11/22/95, Japanese GLP

Vol. 21, pp. 299-322

**Animals :** Male Hartley guinea pigs, 5-8 weeks old, approximately 350-500 g, 5 per group

**Diet:** CG-7 Rodent Chow and water were supplied *ad libitum*.

**Drug Dose, Route of Administration and Sensitization Procedure:** CP-99,219 (trovafloxacin) was suspended in 0.5% methyl cellulose for oral administration or physiological saline for subcutaneous dosing. The trovafloxacin-BSA (bovine serum albumin) and trovafloxacin-OVA (ovalbumin) conjugates were suspended in physiological saline. Animals were sensitized orally or subcutaneously with trovafloxacin (conjugated or not) or BSA as follows:

1. 20 mg/kg trovafloxacin, oral
2. 100 mg/kg trovafloxacin, oral

3. Trovafloxacin plus Freund's Complete Adjuvant (FCA), 10 mg/animal, SC
4. Trovafloxacin-OVA conjugate plus FCA, 1 mg/animal, SC
5. Saline plus FCA, 0.5 ml/animal, SC
6. BSA plus FCA, 1 mg/animal, SC

Guinea pigs were sensitized by oral administration using a dosing schedule of 5 days per week for 3 weeks. Subcutaneous sensitization was accomplished by administering the agents specified above once a week for 4 weeks. Intravenous challenges of 1 mg trovafloxacin or 10 mg trovafloxacin-BSA were given to groups 1-5 19 days after the last oral sensitizing dose or 16 days after the final subcutaneous sensitizing dose. Group 5 (the negative control) was also challenged intravenously with physiological saline and Group 6 (the positive control) was challenged only with 1 mg of BSA. After challenge, the animals were observed for signs of systemic anaphylaxis. Two days prior to the challenge, blood samples were drawn from each of these guinea pigs for use in a passive cutaneous anaphylaxis (PCA) test.

For the latter test, 50  $\mu$ l of a series of diluted sera from the sensitized guinea pigs was injected intradermally into the clipped dorsal skin of a recipient untreated guinea pig. Four hours after injection, either trovafloxacin, trovafloxacin-BSA, saline, or BSA was given to the recipient animals intravenously with Evans blue dye. After 30 minutes, animals were exsanguinated and their skins removed so that the degree of dye extravasation could be measured.

**Results:** It should be noted that IV challenge with trovafloxacin alone caused 2-4 guinea pigs (from groups deemed not to have had anaphylactic reactions) to rub their nose or ears. The investigators called this an "anaphylactic-like sign", but did not consider that animals who demonstrated this behavior in the absence of other clinical signs or symptoms of anaphylaxis as having an anaphylactic reaction. As ear and nose rubbing was also seen in the negative control group, the reviewer suspects that this is a general reaction of guinea pigs to IV trovafloxacin and agrees that it probably not a symptom of even mild systemic anaphylaxis in this case. Systemic anaphylaxis following IV challenge with either trovafloxacin or trovafloxacin-BSA conjugate was not observed in either group of guinea pigs sensitized orally with trovafloxacin, in the animals sensitized with trovafloxacin and FCA subcutaneously, or in the negative control group. Of the guinea pigs sensitized subcutaneously with trovafloxacin-OVA conjugate and FCA, those challenged with IV trovafloxacin did not experience systemic anaphylaxis, but those challenged with IV trovafloxacin-BSA conjugate experienced fatal anaphylaxis. The positive control guinea pigs who were sensitized subcutaneously with BSA plus FCA all exhibited systemic anaphylaxis upon challenge with IV BSA. In that group, 2/5 animals had fatal reactions and the 3/5 had difficulty breathing that lead to cyanosis, but recovered.

Positive cutaneous anaphylaxis did not occur in recipient guinea pigs injected intradermally with serum from negative controls or guinea pigs that had been orally sensitized with trovafloxacin or subcutaneously with trovafloxacin plus FCA. If recipients were injected with serum from animals sensitized with trovafloxacin-OVA, no positive PCA reactions were observed if they were challenged with trovafloxacin alone. Positive PCA reactions were seen in recipients injected intradermally with this serum if trovafloxacin-BSA was used as the challenge agent and PCA titers were . As expected, when recipients were

injected with serum from animals sensitized subcutaneously to BSA with FCA, all exhibited positive PCA reactions with titers of .

Unconjugated trovafloxacin was not antigenic in guinea pigs under the conditions of this assay.

**8-Week Oral (Gavage) Subchronic Study of CP-99,219-27 in Hairless Mice, With or Without Added Simulated Sunlight Protocol No. C-516-001)**

Report dated 3/6/96, U.S. GLP

Vol. 22, pp. 1-198

**Animals:** Male and female Crl:SKH1-hr BR hairless mice, approximately 7 weeks old at the initiation of the study. Gold fluorescent lighting (F40GO) was used in the rooms where mice were housed and exposed to UV to prevent the animals from being exposed to light radiation that could interfere with the study. Each treatment group had 10 mice/sex with an additional 27 mice/sex/group for pharmacokinetic studies of groups 3, 6, and 9-12 and an additional 6 mice/sex/group for pharmacokinetic studies of groups 2 and 8.

**Diet:** Certified Rodent Diet #5002 and tap water purified via reverse osmosis and chlorinated were available *ad libitum* except during UV exposure periods.

**Drug Dose and Route of Administration:** CP-99,219-27 (trovafloxacin mesylate) was suspended in 0.5% carboxymethyl cellulose and administered to mice orally via gavage at dose levels of 0 (vehicle control), 30, 100, 200, or 300 mg/kg/day, 5 days a week (Monday-Friday) at a dose volume of 10 ml/kg. The order of administration of drug and UV exposure was rotated. On Monday, Wednesday and Friday, UV exposure occurred one hour after dosing and on Tuesday and Thursday, drug was administered one hour after UV exposure. Treatment groups were as follows:

	<b>Drug Treatment (mg/kg/day)</b>	<b>Daily UV Exposure (Robertson-Berger Units)</b>
1.	None	0
2.	Vehicle	0
3.	Trovafloxacin, 30	0
4.	Trovafloxacin, 100	0
5.	Trovafloxacin, 200	0
6.	Trovafloxacin, 300	0
7.	None	120
8.	Vehicle	120
9.	Trovafloxacin, 30	120
10.	Trovafloxacin, 100	120
11.	Trovafloxacin, 200	120

12. Trovafloxacin, 300 120

The UV source was a 6.5 KW xenon long arc water cooled burner with a 1 mm thick filter of \_\_\_\_\_ to absorb UVC ( $\leq 290$  nm)- both UVB and UVA were emitted. The dose of UV used in this study was equivalent to 0.3 times the human minimal erythemal dose in untanned human skin (400 Robertson-Berger Units). UV exposure was monitored throughout the study using Robertson-Berger meters mounted on animal racks.

**Length of Study:** Treatment with drug and/or UV occurred 5 days/week (Monday-Friday) for 8 weeks.

**Results:** Mortality was greater than controls in mice receiving 100-300 mg/kg of trovafloxacin. No drug related deaths were observed at 30 mg/kg. The numbers below include deaths or moribund sacrifices believed to be drug-related and not those confirmed to be due to intubation accidents. Most of the drug-related mortality was seen in the first 2 weeks of the study. Data from both genders were combined because there appeared to be no difference between the two. Mortality at these dose levels was also observed in mice from the satellite groups, but they are not included here as the satellite animals were not part of the phototoxicity study, but a separate pharmacokinetic study.

	-UV	+UV	Total
100 mg/kg	3/20	1/20	4/40
200 mg/kg	5/20	3/20	8/40
300 mg/kg	6/20	2/20	8/40

The following intubation accidents were confirmed:

	-UV	+UV
Vehicle	2	
30 mg/kg	1	
100 mg/kg	2	
200 mg/kg		1

APPEARS THIS WAY  
ON ORIGINAL

Necropsy findings from the mice that died on study (death attributed to drug) in the 200 or 300 mg/kg groups included sand-like substance in the cecum, red cecum, dark (brown or black) areas of erosion in fundic mucosa of stomach, hole in the fundic mucosa of stomach, red-brown substance in the stomach, red substance between mucosal layers of cardiac region of stomach, red fundic mucosa of stomach. Another finding observed in the 200 and 300 mg/kg rats, a red or tan to dark brown perianal substance, was also seen in a mouse from the 100 mg/kg group that died on study.

During weeks 1-2 of the study, body weight loss in the 100, 200 and 300 mg/kg male and female mice was significantly ( $p \leq 0.05$ ) greater than controls for the groups without UV exposure. For the groups with UV exposure, body weight loss was significantly less than controls during weeks 1-2 only in the 200 and 300 mg/kg male mice and the 300 mg/kg female mice (2.7 g). The

mean terminal body weight for the male mice in the 300 mg/kg group exposed to UV was significantly less than vehicle control at termination ( $35.2 \pm 2.0$  g vs.  $31.6 \pm 1.7$  g). For the 300 mg/kg males not treated with UV, mean terminal body weight was also less than vehicle control ( $34.7 \pm 2.6$  g vs.  $32.5 \pm 3.7$  g), but the difference was not statistically significant. Mean terminal body weights of female mice were comparable among all of the treatment groups.

With the exception of Grade 1 flaking ("barely perceptible scales) noted during week 1 in a single 300 mg/kg female mouse not exposed to UV, no skin reactions were observed during the study. Trovafloxacin in the presence of simulated sunlight was not phototoxic to hairless mice at doses up to 300 mg/kg for approximately 8 weeks. Trovafloxacin-related mortality was observed at doses  $\geq 100$  mg/kg.

#### Serum Concentrations and Pharmacokinetics of CP-99,219 Following Oral Administration of CP-99,219 to Mice During Toxicology Study C-516-001 (Appendix C)

**Results:** Trovafloxacin concentrations were measured in the serum and skin of satellite groups of hairless mice from the phototoxicity study above. Groups of 3/sex per time point were sacrificed at each time point on study days 3 and 33. Concentrations of trovafloxacin in the serum and skin were measured using a validated

As there were no apparent gender differences, data from male and female animals were combined.

#### Concentration of Trovafloxacin ( $\mu\text{g/ml}$ ) in Serum of Hairless Mice After Oral Administration on Day 3 of Dosing (Mean $\pm$ SD)

Drug Dose (mg/kg)	Predose	Time After Dosing			AUC (0-2) $\mu\text{g}\cdot\text{hr/ml}$
		1 hr	2 hrs	4 hrs	
No UV					
30	BLQ*	$9.1 \pm 2.6$	$4.7 \pm 1.6$	$2.8 \pm 1.2$	6.9
300	$5.0 \pm 1.4$	$23.8 \pm 9.9$	$21.5 \pm 12.2$	$20.2 \pm 5.6$	37.1
+UV					
30	BLQ	$6.5 \pm 2.0$	$6.0 \pm 3.9$	$3.1 \pm 1.4$	6.3
100	$0.7 \pm 0.7$	$15.0 \pm 2.0$	$10.0 \pm 4.7$	$7.8 \pm 2.3$	20.4
200	$6.0 \pm 7.0$	$22.4 \pm 7.3$	$15.7 \pm 4.5$	$12.3 \pm 8.3$	33.3
300	$8.0 \pm 4.4$	$30.3 \pm 9.5$	$23.0 \pm 11.8$	$15.3 \pm 7.1$	45.8

\*Below limit of quantification

**Concentration of Trovafloxacin ( $\mu\text{g/ml}$ ) in Serum of Hairless Mice  
After Oral Administration on Day 33 of Dosing (Mean  $\pm$  SD)**

Drug Dose (mg/kg)	Predose	Time After Dosing		AUC (0-2) $\mu\text{g}\cdot\text{hr/ml}$
		1 hr	2 hrs	
No UV				
30	0.1 $\pm$ 0.1	7.3 $\pm$ 1.3	5.2 $\pm$ 1.4	10.0
300	4.0 $\pm$ 1.3	29.5 $\pm$ 6.2	33.4 $\pm$ 5.0	48.2
+UV				
30	BLQ*	7.3 $\pm$ 2.9	3.2 $\pm$ 1.0	5.3
100	0.3 $\pm$ 0.6	14.6 $\pm$ 3.7	9.6 $\pm$ 7.4	19.6
200	2.3 $\pm$ 1.2	19.8 $\pm$ 4.8	22.5 $\pm$ 12.3	32.2
300	7.1 $\pm$ 7.1	28.8 $\pm$ 7.4	19.9 $\pm$ 8.6	42.3

\*Below limit of quantification

**Concentration of Trovafloxacin ( $\mu\text{g/g}$ ) in Skin of Hairless Mice After Oral  
Administration After 3 or 33 Days of Dosing (Mean  $\pm$  SD)**

Drug Dose (mg/kg)	Day 3			Day 33		
	1 hr	2 hrs	AUC (0-2) $\mu\text{g}\cdot\text{hr/g}$	1 hr	2 hrs	AUC (0-2) $\mu\text{g}\cdot\text{hr/g}$
No UV						
30	5.2 $\pm$ 0.7	4.2 $\pm$ 0.9	7.3	5.9 $\pm$ 0.8	5.1 $\pm$ 0.3	8.5
300	17.3 $\pm$ 2.9	14.9 $\pm$ 3.9	24.8	22.3 $\pm$ 2.7	20.7 $\pm$ 4.2	32.7
+ UV						
30	4.3 $\pm$ 0.8	4.1 $\pm$ 2.0	6.4	6.4 $\pm$ 1.5	4.3 $\pm$ 0.8	8.6
100	9.9 $\pm$ 1.1	10.3 $\pm$ 6.9	15.1	10.9 $\pm$ 2.3	8.4 $\pm$ 3.4	15.1
200	15.1 $\pm$ 2.5	13.5 $\pm$ 4.6	21.9	19.9 $\pm$ 5.1	16.1 $\pm$ 8.3	28.0
300	19.4 $\pm$ 2.0	18.3 $\pm$ 9.2	28.6	16.4 $\pm$ 3.1	19.3 $\pm$ 5.5	26.1

Serum and skin levels of trovafloxacin increased with dose in hairless mice. At the sampling times of 1 and 2 hours after administration, there was usually more drug in serum than in skin. The skin/serum C<sub>max</sub> ratios on day 3 ranged from \_\_\_\_\_ and those on day 33 ranged from \_\_\_\_\_. The skin/serum AUC<sub>0-2 hr</sub> ratios determined on day 3 ranged from \_\_\_\_\_ and those on day 33 ranged from \_\_\_\_\_. Exposure to UV radiation did not appear to have an effect on the serum or skin levels of trovafloxacin.

**CP-99,219-27 Exploratory Neonatal Study in Sprague-Dawley Rats Dose Levels: 3, 6, 12, or 25 mg/kg (Study No. 95-783-30)**

M.S. Tassinari (Reproductive and Developmental Toxicology, Pfizer, Groton, CT)

Report not dated, Not GLP

Vol. 25, pp. 1-17

**Animals:** Timed pregnant female Sprague-Dawley rats were received on day 16 of gestation. Eight littered on the same day and their offspring were used for the study. Viable pups were weighed and sexed on postnatal day 1 and randomly distributed among the dams to form litters of 5 per sex containing no more than one male and one female pup that had come from the same original litter. Each dose group had 2 litters.

**Diet:** Dam's diet not specified; neonates consume dam's milk only.

**Drug Dose and Route of Administration:** Trovafloxacin was administered once daily to neonates via oral gavage (vehicle was 0.5% methylcellulose) at doses of 3, 6, 12, or 25 mg/kg. Dams were not treated with drug.

**Length of Study:** Pups were treated with drug on postnatal days 4-7. Pups were bled 0.5, 1, or 2 hours after the last dose for determination of serum trovafloxacin. Brains were harvested from the pups at the same time, also for measurement of drug. The method for determining the serum and brain levels of trovafloxacin was not included in this report.

**Results:** The purpose of the study was to find the NOEL for tremors in neonate rats dosed with trovafloxacin. Intermittent whole body tremors were observed in pups treated with 25 mg/kg trovafloxacin beginning 1-2 hours after dosing. Tremors were no longer observed in most of these pups 6 hours after administration of the drug. Tremors were not observed in any of the pups from the 3, 6, or 12 mg/kg dose groups.

Trovafloxacin was found in the brains of the pups and the mean brain/serum ratios were 0.8 at C<sub>max</sub> and 0.8 for AUC. Both serum and brain levels of trovafloxacin were relatively constant over the sampling period within each dose level. Concentrations of trovafloxacin in serum and brain increased in a dose-dependent manner that was close to linear.

**Serum Levels of Trovafloxacin ( $\mu\text{g/ml}$ ) in Neonatal Rats  
After 4 Daily Doses (Mean  $\pm$  SD)**

Dose (mg/kg)	Time After Dosing (hours)			AUC (0-2) $\mu\text{g}\cdot\text{hr/ml}$
	0.5	1.0	2.0	
3	0.7 $\pm$ 0.3	0.6 $\pm$ 0.2	0.9 $\pm$ 0.2	1.3
6	1.6 $\pm$ 0.1	1.6 $\pm$ 0.2	1.5 $\pm$ 0.6	2.8
12	4.0 $\pm$ 1.3	4.0 $\pm$ 1.6	4.0 $\pm$ 0.5	7.0
25	7.9 $\pm$ 1.3	7.4 $\pm$ 1.7	7.3 $\pm$ 1.1	13.2

**Brain Levels of Trovafloxacin ( $\mu\text{g/g}$ ) in Neonatal Rats After 4 Daily Doses (Mean  $\pm$  SD)**

Dose (mg/kg)	Time After Dosing (hours)			AUC (0-2) $\mu\text{g}\cdot\text{hr/g}$
	0.5	1.0	2.0	
3	0.5 $\pm$ 0.3	0.7 $\pm$ 0.2	0.6 $\pm$ 0.3	1.1
6	1.0 $\pm$ 0.2	1.2 $\pm$ 0.3	1.1 $\pm$ 0.4	2.0
12	2.1 $\pm$ 0.5	3.4 $\pm$ 1.2	2.8 $\pm$ 0.5	5.0
25	4.8 $\pm$ 1.1	6.3 $\pm$ 1.5	5.1 $\pm$ 1.2	9.7

Tremors in neonatal rats were observed after dosing with 25 mg/kg of trovafloxacin, but not 12 mg/kg; thus the threshold dose is somewhere between. The investigators mentioned that tremors were observed sporadically in neonatal rats dosed with 15 mg/kg for an earlier study; this is consistent with the results observed in the current study. The earlier study also demonstrated that rat pups more than 22 days old were not as sensitive as younger pups to the induction of tremors and their brain tissue had lower levels of trovafloxacin than younger pups given the same oral dose. The investigators believe that the blood brain barriers were more developed in the older pups; this is a reasonable assumption. In the current study conducted on 3-7 day old rat pups, brain levels of trovafloxacin  $\leq 3.4 \mu\text{g/g}$  were not associated with tremors, but brain levels of trovafloxacin  $> 4.8 \mu\text{g/g}$  were. These toxicokinetic data were consistent with previous studies in rat pups.

**A Two-Week Range Finding Study of CP -99,219-27 in Neonatal Beagle Dogs Dose Levels 7.5, 15.0 (7.5 BID), 50.0 (25 BID) mg/kg/day (Study No. 94461)**

Report dated 3/22/95, U.S. GLP

Vol. 25, pp. 18-102

**Animals:** Neonatal beagle pups were selected for the study within 48 hours of birth and two per sex were assigned to treatment groups in a fashion to ensure that litter mates were not assigned to the same group. It was not clear whether one dam nursed all 4 members of a treatment group.

**Diet:** Pups nursed their mothers *ad libitum* except for one hour before dosing. A milk replacement was given to pups that were not nursing adequately. One pup in the 15 mg/kg group was fed milk replacer for one day, and all 4 pups in the 50 mg/kg group were fed the replacer for 1, 4 (2 pups) or 7 days between days 4-11 of the study. The dams were given certified and tap water *ad libitum*.

**Drug Dose and Route of Administration:** Trovafloxacin was suspended in 0.5% methyl cellulose at concentrations of 1.5 and 5 mg/ml and administered orally via gavage at a dose volume of 5 ml/kg. Pups were removed from their mothers one hour prior to dosing, then returned after the dose of drug was administered. Dose groups were as follows:

1. Control (vehicle, 5 ml/kg b.i.d.)
2. 7.5 mg/kg/day
3. 15 mg/kg/day (7.5 mg/kg b.i.d., 4 hours apart)
4. 50 mg/kg/day (25 mg/kg b.i.d., 4 hours apart)

**Length of Study:** The duration of dosing was 15, 18, or 21 days. Dosing began on the same day for all animals and continued until an animal was sacrificed. Members of one dose group were not all sacrificed on the same day. For toxicokinetic analysis, blood samples were drawn 1, 4, and 12 hours after the first and last doses of drug.

**Results:** None of the 50 mg/kg pups survived for the entire study. They were sacrificed in moribund condition on days 5-10 of dosing. The 50 mg/kg pups did not nurse and were hand fed a milk replacer, but they had diarrhea and became dehydrated. Emesis was seen in 2 of the high dose pups. All of the high dose pups were less active than those from the other dose groups and one was described as "lethargic" for 2 days prior to sacrifice. One female in the 15 mg/kg group experienced excessive salivation on day 5 of dosing and had some difficulty breathing on days 5 and 6 of dosing. One female in the control group died from a gavage accident on day 14 of dosing. Soft to watery feces were sporadically observed in pups from the 7.5 and 15 mg/kg groups on days 12-18 of dosing. The investigators described the change in fecal consistency as "not unusual for colony dogs at this age", but none of the control animals had soft or watery feces, so it may have been a drug-related effect.

Body weight gain in animals treated with 7.5 mg/kg of trovafloxacin did not appear to be different from control, but it was modestly suppressed in the 15 mg/kg group and severely suppressed in the 50 mg/kg group.

Treatment of beagle neonates with up to 15 mg/kg trovafloxacin did not appear to affect the attainment of developmental landmarks such as eye and ear opening. As the scheduled sacrifices of the surviving animals began around the time that the pups leave the

pack and tooth eruption occurs, some were sacrificed before these landmarks had been attained. However, there appeared to be no sign of developmental delay at doses of trovafloxacin up to 15 mg/kg/day.

Drug-related changes in hematologic and clinical chemistry parameters were not observed at doses of trovafloxacin up to 15 mg/kg/day. A drop in the number of white blood cells was observed in one male and one female dog in the high dose group at the time of sacrifice. A drop in the number of red blood cells was observed in all of the dogs in the high dose group when they were sacrificed, especially the two females. One high dose female dog also had a very low number of platelets. As these dogs were malnourished and in poor condition at the time of sacrifice, it is difficult to determine whether these changes were due to drug or were secondary to the drug-related lack of nourishment.

No drug-related gross or microscopic findings were observed in the dogs dosed with 75 or 15 mg/kg of trovafloxacin. Absolute organ weights tended to be lower than control in both of these groups; but relative organ weights were similar. Microscopic findings in the tissues of the pups from the 50 mg/kg dose group did not appear to be directly related to drug treatment, but were consistent with malnutrition. These findings included reduced glycogen stores in hepatocytes, depletion of hematopoietic stem cells in marrow of rib, sternum and femur, lymphoid depletion of tonsil, thymus, and spleen, and reduced bone growth (including reduction in size or number of osteoblasts in rib, sternum, femur, humerus) It is difficult to determine whether vacuolar changes in the lenticular fibers of the eye observed in 3/4 50 mg/kg pups were related to trovafloxacin dosing or to the milk replacement given from 4-7 days. The pup in this dose group that received only one day of milk replacer was the one who did not exhibit changes in the eye. Some quinolones have been associated with cataracts in dogs, but subcapsular cataracts have also been associated with milk replacers in orphaned puppies, perhaps due to a nutritional deficiency that has not been definitively identified. Finally, fungal infection of the GI mucosa was observed in one high dose female.

Concentrations of trovafloxacin in serum collected on the first and last days of dosing were measured. On study day 1, the  $AUC_{0-12\text{ hr}}$  increased in a close to linear fashion in accordance with the total daily dose. There is evidence of drug accumulation at 15 mg/kg/day.

**Serum Concentrations ( $\mu\text{g/ml}$ ) of Trovafloxacin in Beagles  
After the First and Last Doses (Mean  $\pm$  SD)**

	7.5 mg/kg	15 mg/kg	50 mg/kg
<b>First Dose</b>			
<b>1.0 hours</b>	1.4 + 0.5	1.2 + 0.9	4.2 + 1.2
<b>4.0 hours</b>	1.1 + 0.5	1.4 + 0.4	3.7 + 1.3
<b>12.0 hours</b>	0.7 + 0.4	1.7 + 0.5	5.2 + 1.9
<b><math>AUC_{0-12\text{ h}}</math> (<math>\mu\text{g}\cdot\text{hr/ml}</math>)</b>	11.6 + 4.8	16.7 + 3.5	49.6 + 10.7

<b>Last Dose</b>			
<b>1.0 hours</b>	1.4 + 0.9	3.1 + 1.1	---
<b>4.0 hours</b>	1.2 + 0.7	2.2 + 0.5	---
<b>12.0 hours</b>	0.6 + 0.4	1.9 + 0.4	---
<b>AUC<sub>0-12 h</sub> (µg·hr/ml)</b>	12.0 + 7.6	25.9 + 3.3	---

Pharmacokinetic data not available for the last day of dosing of the 15 mg/kg animals as all were sacrificed in moribund condition.

Neonatal beagle pups tolerated up to 15 mg/kg (7.5 mg/kg, b.i.d.) of trovafloxacin for 15-21 days with minimal signs of toxicity (slight suppression of body weight gain, 2 episodes of difficult respiration in one animal), but 50 mg/kg (25 mg/kg, b.i.d.) was very toxic. None of the neonatal beagles dosed with 50 mg/kg survived past day 10 and signs of malnourishment secondary to a drug-related failure to nurse were seen in all animals from this dose group. Feeding a milk replacer to the pups failed to prevent the malnourishment.

**CP-116,517-27 A Single Dose Oral Toxicity Study in Rats, and A Single Dose Dermal Toxicity Study in Rabbits, and A Single Dose Ocular Irritation Study in Rabbits (Hazard Assessment Testing) (Study No. 95-851-14)**

N.J. Johnson (Pfizer Central Research, Groton, CT)

APPROPRIATE WAY  
ON ORIGINAL

Report dated 10/9/95, U.S. GLP

Vol. 26, pp. 34-49

**Summary and Results:** Studies were designed according to U.S. Department of Transportation guidelines so that hazard classification could be designated.

For the oral dosing study, alatrofloxacin was dissolved in deionized water at a concentration of 100 mg/ml and a single dose (2000 mg/kg, 20 ml/kg) was given to 3 male and 3 female Crl:CD BR, VAF/Plus rats. One female rat died on day 3 after dosing. Before death, it was cold to the touch, appeared weak, vocalized when handled, had decreased respiration rate, soft stool, and exhibited slight pilomotor erection. The remaining 5/6 rats survived for the entire 14 day post dose observation period. One showed no clinical symptoms after dosing, the others showed decreased activity. No gross tissue changes were observed upon necropsy.

For the dermal study, 2000 mg of alatrofloxacin powder was applied to intact skin of 4 male and 1 female New Zealand White rabbits and placed under an occluded dressing for 24 hours. Skin was premoistened with normal saline. None of the animals died during the 14 day observation period following the application of the test material and no clinical signs of toxicity were observed with the exception of reduced food consumption and a concomitant reduction in the size and number of fecal pellets. Very slight erythema was observed on the

skin of 2/5 rabbits after the 24 hour drug exposure period, but this resolved completely by the next day and no other skin changes were seen throughout the remainder of the study.

For the eye irritation study, 20.1 mg of alatrofloxacin powder was applied to the conjunctival sacs of the left eyes of 1 male and 2 female New Zealand White rabbits. The eyes were not rinsed after application and the animals were observed for 4 days. Eyes were observed 1, 5, and 24, 48, and 72 hours after dosing. None of the eyes exhibited corneal opacity or iritis at any time up to 4 days after alatrofloxacin powder was applied. Slight redness of the palpebral conjunctiva, slight chemosis, and a moderate amount of a clear, colorless discharge were observed in all eyes 1 and 5 hours after application of alatrofloxacin powder. Twenty-four hours after dosing, only a slight redness of the palpebral conjunctiva was observed in the treated eyes. All eyes were normal by 72 hours after dosing.

Alatrofloxacin is not poisonous, corrosive, or an eye irritant according to DOT guidelines.

#### **Reproduction Studies:**

##### **CP-99,219-27 Oral Teratology Study (Study III) in Sprague-Dawley Rats Dose Levels 5, 15 and 75 mg/kg/day (Study No. 93-01-52)**

M. Horimoto (New Product Development Center, Pfizer Pharmaceuticals, Inc., Aichi, Japan)

Report dated 1/28/94, Japanese GLP

Vol. 26, pp. 158-202

**Animals:** Female Sprague-Dawley Rats (177-218 g on the first day of mating) were mated with males of the same strain (319-359 g on the first day of mating); group assignments made after females were sperm-positive so that each treatment group would have a similar mean body weight. Rats were approximately 10 weeks old at the initiation of the mating period. Each treatment group had 20 females. Males were mated with more than one female, as there were a total of 40 males available for the study. The report does not specify which male was mated to a particular female.

**Diet:** Pelleted commercial laboratory animal chow and tap water were provided *ad libitum*.

**Drug Dose and Route of Administration:** CP-99,219-27 (trovafloxacin mesylate) was suspended in 0.5% aqueous methyl cellulose and administered via oral gavage once daily for 12 days (days 6-17 of gestation) to sperm-positive, presumed pregnant female rats at a dose volume of 20 ml/kg. Treatment groups were as follows:

1. Vehicle control
2. 5 mg/kg
3. 15 mg/kg
4. 75 mg/kg

**Length of Study:** Presumed pregnant females were treated from days 6-17 of gestation and the rats assigned to the reproductive toxicity study groups were sacrificed on day 21. Half of the fetuses were fixed in Bouin's solution and examined for visceral malformations after sections were cut with a razor blade. The other half were stained with alizarin red S and examined for skeletal development and malformations.

**Results:** None of the dams died during the course of the study and no overt clinical signs of toxicity were observed. One of the rats in the 5 mg/kg group was not pregnant. Mean food consumption and body weights were similar for control and treated groups of dams. Necropsy did not reveal any gross drug-related changes in the dams.

The number of corpora lutea per dam did not differ among the treatment groups, nor would one expect it to since drug treatment did not commence until day 6 of gestation. Dosing pregnant female rats with up to 75 mg/kg of trovafloxacin did not appear to have a biologically significant effect on-preimplantation or postimplantation loss.

The mean number of live fetuses per litter did not differ significantly among the treatment groups and mean fetal and placental weights were similar. Trovafloxacin-related external or visceral abnormalities were not observed at dose levels up to 75 mg/kg. An increased incidence of fetuses with skeletal variations (presence of cervical rib and/or shortened 13th rib) was observed in the 75 mg/kg dose group. Additionally, the percentage of fetuses in the 75 mg/kg group with rudimentary 5th sternebra was significantly greater than control ( $p < 0.001$ , 54.8% vs. 9.2%). An increase in the percentage of fetuses with rudimentary 5th sternebra was also observed at 15 mg/kg (15.6%), and this was suggestive of a dose-response relationship although the increase was not statistically significant. The number of ossified caudal vertebrae and right and left phalanges was significantly ( $p < 0.001$ ) reduced in the 75 mg/kg group compared to control and the number of ossified caudal vertebrae was slightly, but significantly ( $p < 0.05$ ) reduced in the 15 mg/kg fetuses (control:  $8.7 \pm 0.7$ , 15 mg/kg:  $8.2 \pm 0.7$ , 75 mg/kg:  $7.0 \pm 0.9$ ). The skeletal effects seen at 75 mg/kg are consistent with the increase in variations observed in other rat teratology studies with trovafloxacin or alatrofloxacin.

The NOEL for dams in this study was 75 mg/kg and the NOEL for fetuses was 7.5 mg/kg. It is difficult to dismiss the findings of delayed ossification at 15 mg/kg, although they are slight, due to the apparent dose-response relationship for the number of fetuses with rudimentary 5th sternebra and the reduction in the number of ossified caudal vertebrae. The reviewer believes that the skeletal variations seen in the fetuses at 75 mg/kg demonstrate evidence of teratogenicity.

**CP-99,219-27 Reproductive Study II Prenatal and Postnatal Development in Sprague-Dawley Rats Dose Levels 5, 15 and 75 mg/kg/day (Study No. 94-783-18)**

W.P. Weisenburger (Pfizer Central Research, Groton, CT)

Report dated 11/27/95, U.S. GLP

Vol. 27, pp. 1-308