CENTER FOR DRUG EVALUATION AND RESEARCH

APPLICATION NUMBER:

21-275

PHARMACOLOGY REVIEW

REVIEW AND EVALUATION OF PHARMACOLOGY/TOXICOLOGY DATA

KEY WORDS: Lumigan™, bimatoprost ophthalmic solution, 0.03%, AGN 192024, intraocular pressure (IOP), ocular hypertension, glaucoma

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IND/NDA number: **NDA 21-275**
Serial number/date/type of submission: 000/September 18, 2000/commercial
Information to sponsor: Yes (X), No ( )
Sponsor: Allergan
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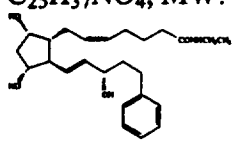
Drug:

Generic Name: **Bimatoprost**
Code Name: **AGN 192024**
Trade Name: Lumigan
Chemical Name: (Z)-7-[(1R, 2R, 3R, 5S)-3, 5-Dihydroxy-2-[(1E, 3S)-3-hydroxy-5-phenyl-1-pentenyl] cyclopentyl]-N-ethyl-5-heptenamide
CAS Registry Number: 155206-00-01
Molecular Formula: $C_{25}H_{37}NO_4$, MW: 415.58



Dosage Form: Ophthalmic solution, 0.03%
Proposed Dose: One drop (30 µl) in the affected eye, once daily in the evening (Total dose could be 0.018 mg/patient/day or 0.0003 mg/kg/day for a 60 kg adult)

Relevant INDs/NDAs/DMFs: [redacted]
Drug Class: $PGF_{2\alpha}$ analog
Clinical Formulation:

Ingredients	Function	Concentration (% w/v)	Concentration (mg/ml)
Bimatoprost (AGN 192024)	Active ingredient	[redacted]	0.3
Benzalkonium chloride, NF			0.05
Sodium phosphate			
Citric acid			
Sodium chloride USP			
Hydrochloric acid NF			
Sodium hydroxide NF			
Purified water USP			

Indication:	Reduction of elevated intraocular pressure in patients with glaucoma or ocular hypertension
Route of administration:	Ocular, Topical

Proposed clinical protocol or use:

AGN 192024 0.03% ophthalmic solution is for lowering the elevated IOP in patients with glaucoma or ocular hypertension. The recommended dose is one drop instilled into each affected eye, once daily in the evening.

Previous clinical experience:

The sponsor has conducted a series of clinical studies in patients with glaucoma or ocular hypertension to evaluate the safety and efficacy of AGN 192024 ophthalmic solution at various concentrations and dosing regimens. AGN 192024 at concentrations of 0.01%, 0.03% and 0.1%, administered qd and/or bid, was shown to be an effective ocular hypotensive agent, and had an acceptable safety profile. The most frequently reported adverse events were conjunctival hyperemia, growth of eyelashes and eye pruritus. No serious treatment-related adverse events were reported.

Introduction and drug history:

AGN 192024 is a synthetic analog of prostaglandin $F_{2\alpha}$. The drug is different from prostaglandin in that it does not stimulate any previously described prostanoid receptors. Animal studies have indicated that AGN 192024 is a potent ocular hypotensive agent. The drug is under development by Allergan as an ophthalmic formulation for the treatment of glaucoma.

NONCLINICAL STUDIES:

Pharmacology:

1. Studies on the effects of AGN 192024 on the beagle dog eye. Report #: BIO-94-062, Vol. 12, Page 001

The purpose of these studies was to determine the effects of AGN 192024 on the beagle dog eye following 5-day administrations (bid, ocular topical) with 0.001%, 0.01% and 0.1% concentrations of AGN 192024. A significant and well-maintained reduction in IOP was produced by all 3 doses of AGN 192024. The effects of the 0.01% and 0.1% doses on dog IOP were very substantial and exceeded a 40% decrease in IOP. AGN 192024 also produced miosis but the effect did not persist over a 24 hr period.

In terms of IOP and miotic effects, AGN 192024 showed similar potency to PGF $_{2\alpha}$-1-isopropyl ester (a potent ocular hypotensive agent in beagle dogs) and latanoprost. Comparison of ocular surface hyperemia showed differences among the 3 compounds. At a 0.01% dose the level of ocular hyperemia for AGN 192024 was very slight compared to slight to moderate responses for latanoprost and moderate to severe responses for $PGF_{2\alpha}$-1-isopropyl ester. These results suggested that AGN 192024 might be a clinically potent ocular hypotensive with minimal ocular surface hyperemia. The sponsor indicated that the miotic effect could be feline and canine specific and unlikely to be manifested clinically.

2. AGN 192024 does not increase outflow facility in the beagle dog and the potent ocular hypotensive activity is not cyclooxygenase dependent. Report #: BIO-94-065, Vol. 12, Page 112

The purpose of this study was to determine the effects of AGN 192024 on outflow facility in the beagle dog eye. AGN 192024 was compared with $PGF_{2\alpha}$-1-isopropyl ester in this study. Both compounds decreased IOP with rapid onset, good potency (↓8-12 mmHg) and long duration (> 24 hr). $PGF_{2\alpha}$-1-isopropyl ester increased outflow facility in the test eye from 0.39 µl/min/mmHg to 1.89 µl/min/mmHg, while AGN 192024 did not affect outflow facility. Pretreatment of eyes with [redacted] a COX inhibitor, slightly slowed the rapid IOP decrease to AGN 192024. In summary, AGN 192024 had no effect on outflow facility in the beagle dog eye. The hypotensive activity was probably due to improved aqueous humor drainage via the uveoscleral pathway. The large and sustained IOP decrease to AGN 192024 did not involve a COX-dependent mechanism.

3. Studies on the effects of ocular hypotensive lipids AGN 192024 and AGN 192151, administered once daily, on beagle dog eyes. Report #: BIO-96-112, Vol. 12, Page 228

The purpose of these studies was to determine the effects of AGN 192024 and AGN 192151 on the beagle dog IOP, pupil diameter (PD) and ocular surface hyperemia (OSH) following 5-day administrations (qd, ocular topical) with 0.01% and 0.1% concentrations of AGN 192024 and AGN 192151. These 2 compounds were compared with latanoprost. Both AGN 192024 and AGN 192151 showed a decrease in IOP at all doses while AGN 192024 appeared 10 times more potent than AGN 192151. In terms of miotic effects, all compounds showed similar potency. Comparison of ocular surface hyperemia showed differences. At a 0.1% dose the levels of ocular hyperemia for AGN 192024 and AGN 192151 were slight while the hyperemia was slight to moderate for latanoprost. These results suggested that AGN 192024 might be a clinically potent ocular hypotensive with minimal ocular surface hyperemia.

4. Further studies on the effects of ocular hypotensive lipids AGN 192024, administered once daily, on beagle dog eyes. Report #: BIO-99-318, Vol. 14, Page 001

The purpose of these studies was to determine the effects of AGN 192024 on the beagle dog IOP following 5-day administrations (qd, ocular topical) with 0.003%, 0.006% and 0.03% concentrations of AGN 192024. At all 3 concentrations, AGN 192024 produced statistically significant, well-maintained reductions in IOP (↓ up to 3.1-6.2 mmHg). AGN 192024 also produced miosis but this effect did not persisted over a 24 hr period. Trace to mild ocular surface hyperemia was observed at all 3 concentrations.

AGN 192024 0.03% was also compared with latanoprost 0.005%. Both compounds showed a decrease in IOP while AGN 192024 appeared more efficacious than latanoprost with IOP levels 0.5-1 mmHg lower on Days 4 and 5. In terms of miosis and hyperemia, both compounds showed similar responses. These results suggested that AGN 192024 might be a clinically potent ocular hypotensive with minimal ocular surface hyperemia.

5. Studies on the effects of AGN 192024 on the cynomolgus monkey eye. Report #: BIO-94-067, Vol. 12, Page 126

The purpose of these studies was to determine the effects of AGN 192024 on the female cynomolgus monkey eye following 5-day administrations (bid, ocular topical) with 0.001%, 0.01% and 0.1% concentrations of AGN 192024. A significant and well-maintained reduction in IOP was produced by all 3 concentrations of AGN 192024 in both ocular normotensive monkeys (↓3-4 mmHg) and laser-induced ocular hypertensive monkeys (↓7-13 mmHg). AGN 192024 had no effects on aqueous humor inflow. AGN 192024 did not result in miosis.

6. Effects of AGN 192024 in aqueous humor outflow in monkeys. Report #: BIO-99-323, Vol. 14, Page 088

The purpose of this study was to determine the effects of AGN 192024 on outflow facility and on uveoscleral outflow in cynomolgus monkey eye following 5-day administrations (bid, ocular topical) with 0.01% AGN 192024. Aqueous humor conventional outflow facility was determined using the 2-level constant pressure perfusion method. Uveoscleral outflow was determined using an intracameral fluorescent tracer. The results showed that AGN 192024 0.01% increased uveoscleral outflow by approximately 42%. In conclusion, in cynomolgus monkeys, AGN 192024 appeared to decrease IOP by stimulating uveoscleral outflow.

7. The effects of AGN 192024 0.03% on the intraocular pressure of laser-induced ocular hypertensive cynomolgus monkeys. Report #: BIO-00-354, Vol. 14, Page 243

The purpose of these studies was to determine the effect of AGN 192024 0.03% on IOP changes 1 to 6 hr following a sing ocular administration of the drug to laser-induced, unilaterally ocular hypertensive cynomolgus monkeys. The results showed that the IOP in the ocular hypertensive eyes was reduced from 42.5 mmHg to 26.5 mmHg (↓34.9%). This study supported AGN 192024 as a potent ocular hypotensive agent.

8. The pharmacology of AGN 192024. Report #: BIO-94-068, Vol. 12, Page 153

9. The pharmacology of AGN 192024. Report #: BIO-95-085, Vol. 12, Page 180

The purpose of these studies was to determine the pharmacological profile by using a wide variety of prostanoid receptor assays. A battery of prostanoid receptor assays showed that the activity of AGN 192024 at these receptors described in the current classification (DP, EP1-3, FP, IP and TP) was either absent or trivial. AGN 192024 was highly selective for stimulating 2 $PGF_{2\alpha}$-sensitive preparations, the cat iris and the cat lung parenchyma. However, AGN 192024 did not stimulate the FP receptor in Swiss 3T3 cells or bind to the cloned human FP receptor transiently expressed in COS-7 cells. It appeared that AGN 192024 exerted its effects by stimulating a receptor that was pharmacologically distinct from the FP or any other known prostanoid receptors.

10. General pharmacological study of AGN 192024. Report #: BIO-00-355, Vol. 14, Page 252

This general pharmacological study was conducted in mice, rats, guinea pigs, rabbits and dogs. Intravenous administration was selected for the in vivo testing. In addition, eye drops were selected in the corneal reflex test in guinea pigs.

AGN 192024 showed no effects in the general activity and behavior tests in rats, even at 1 mg/kg. In the tests evaluating drug's effects on CNS, which included spontaneous locomotor activity and hexobarbital-induced sleeping in rats, the pentetrazol-induced convulsions and [redacted] method-induced pain test in mice, and rectal temperature measurement in rats, AGN 192024 demonstrated no effects, even at 1 mg/kg. In respiratory and cardiovascular systems in conscious dogs, AGN 192024 transiently increased blood pressure (↑9-26%) at 10 μg/kg. In the digestive system, AGN 192024 inhibited the small intestinal charcoal transit (↓25%) in rats at 1 mg/kg, but it did not affect intestinal water pooling in rats at the same dose. AGN 192024 increased urine volume (↑16%) and excretion of urinary electrolytes (Na^+, 49% and Cl^-, 33%) at 1 mg/kg. AGN 192024 0.1% eye drops showed no effects on corneal reflex in guinea pigs.

In in vitro assays, AGN 192024 at concentrations up to 2×10^{-6} M had no effects on acetylcholine-, histamine-, barium chloride- or serotonin-induced contraction in isolated guinea pig ileum or spontaneous motility in non-pregnant and pregnant rat uterus. AGN 192024 at 2×10^{-7} M and 2×10^{-6} M increased contractile force of uterus specimens in non-pregnant rabbits.

In summary, AGN 192024 showed some undesirable pharmacological effects in certain assays. However, these effects occurred at very high dose levels or concentrations. These dose levels and concentrations were not likely to be used in clinical treatment.

1. The pharmacology of prostaglandin $F_{2\alpha}$ 1-ethanolamide. Report #: BIO-99-308, Vol. 13, Page 064

$PGF_{2\alpha}$ 1-ethanolamide was recently discovered present in tissues from naïve mice. The studies listed in this report were performed to determine whether $PGF_{2\alpha}$ 1-ethanolamide might act as a biologically active hormone.

$PGF_{2\alpha}$ 1-ethanolamide was very potent in stimulating cat iris sphincter smooth muscle. This effect did not appear to involve the $PGF_{2\alpha}$-sensitive FP receptor since $PGF_{2\alpha}$ 1-ethanolamide exhibited relatively modest affinity for the cat and human FP receptor.

No activity at DP and IP receptors was apparent. Residual interaction was apparent in the guinea pig ileum and rat aorta, which were indicative of EP_1 and TP receptor interaction, respectively. Activity in EP_3 receptor preparations was more pronounced in the chick ileum and guinea pig vas deferens preparations. Compared with $PGF_{2\alpha}$ 1-ethanolamide, AGN 192024 was more selective and had minimal activity at EP_3 receptors.

$PGF_{2\alpha}$ 1-ethanolamide was an ocular hypotensive agent in dogs with miotic effects and mild hyperemia effects.

In summary, $PGF_{2\alpha}$ 1-ethanolamide appeared to be a naturally occurring hormone with unique pharmacology. The similarities between AGN 192024 and $PGF_{2\alpha}$ 1-ethanolamide

suggested that AGN 192024 might be a compound that mimics the activity of the natural hormone $PGF_{2\alpha}$ 1-ethanolamide.

12. Biosynthesis of $PGF_{2\alpha}$ 1-ethanolamide from anandamide using cyclooxygenase-2 and prostaglandin F synthase in vitro. Report #: PK-99-053, Vol. 45, Page 108

The purpose of this in vitro study was to determine if $PGF_{2\alpha}$ 1-ethanolamide was a metabolite of anandamide. 70 mM of ^{3}H-anandamide was incubated with 17-1000 units/ml of ovine cyclooxygenase-2 at 37 °C for 20 min. The incubation samples were extracted and analyzed with [redacted] The formation of $PGF_{2\alpha}$ 1-ethanolamide catalyzed by COX-2 alone was minimal with the highest yield of $PGF_{2\alpha}$ 1-ethanolamide of 2.33% (1.68 nmol). When anandamide (70 µM) was incubated with 33 units/ml COX-2 and 50 µl/ml of E. coli expressing bovine lung PGF synthase, the average amounts of $PGF_{2\alpha}$ 1-ethanolamide produced were 3.1% (2.2 nmol) at 5 min, 0.74% (0.53 nmol) at 20 min and 2.1% (1.5 nmol) at 40 min. In conclusion, $PGF_{2\alpha}$ 1-ethanolamide was a metabolite of anandamide catalyzed by COX-2 and PGF synthase. The enzymatic formation of $PGF_{2\alpha}$ 1-ethanolamide from anandamide appeared analogous to the formation of $PGF_{2\alpha}$ from arachidonic acid.

13. Metabolic profile of anandamide and formation of prostaglandin $F_{2\alpha}$ 1-ethanolamide in mice following a single intravenous administration of ^{3}H-anandamide. Report #: PK-99-052, Vol. 45, Page 087

Anandamide, an endogenous ligand of cannabinoid receptors, is widely distributed in the body with high concentrations in the brain tissues. The purpose of this study was to examine the metabolism and formation of $PGF_{2\alpha}$ 1-ethanolamide in male Swiss Webster mice following a single iv administration of ^{3}H-anandamide (100 mg/kg). Blood, lung and liver samples were collected at 5 and 30 min after dosing. The samples were analyzed by [redacted]

The results are summarized in the table below. Five min after dosing with ^{3}H-anandamide, many metabolites including arachidonic acid, $PGF_{2\alpha}$ 1-ethanolamide, $PGE_{2\alpha}$ 1-ethanolamide, $PGF_{2\alpha}$, PGH_2 and some unknown polar metabolites were detected in the lung, liver and blood, indicating anandamide was rapidly metabolized in mice. $PGF_{2\alpha}$ 1-ethanolamide was an endogenous compound and also a metabolite of anandamide.

Percent of total radioactivity of ^{3}H-anandamide and its metabolites in mice

	Ananda mide	$PGF_{2\alpha}$ 1-ethanolamide	$PGE_{2\alpha}$ 1-ethanolamide	$PGF_{2\alpha}$	PGE_2	PGD_2	PGH_2	Arachidonic acid	Unknown
Lung									
5 min	30.2	5.86	1.28	0.204	Not detected	≤ 0.080	4.31	11.3	46.3
30 min	7.67	2.14	Not detected				4.63	38.3	46.6
Liver									
5 min	5.47	2.01	0.683	1.24	0.282	1.75	1.07	31.9	55.4
30 min	0.897	3.25	≤ 0.424	0.425	0.333	1.22	1.22	40.9	53.8
Blood							-		
5 min	59.0	1.75	≤ 1.36	1.33	≤ 0.230	0.410	1.05	6.95	29.0
30 min	79.1	Not detected							20.9

14. Interaction of AGN 192024 and AGN 192151 with recombinant prostanoid receptors. Report #: BIO-99-313, Vol. 13, Page 156

The purpose of this study was to determine the effects of AGN 192024 and AGN 192151 on selected prostanoid receptors (recombinant human EP_2, EP_3, EP_4, TP and FP receptors, feline FP receptor). Despite substantial overexpression of both human and feline FP receptors, AGN 192024 remained very weak compared to 17-phenyl-$PGF_{2\alpha}$. AGN 192024 at 10^{-8} M and 10^{-7} M concentrations, which consistently stimulated the cat iris and lung parenchyma, did not stimulated the overexpressed recombinant feline and human FP receptors. Similar to FP receptor, AGN 192024 showed residual activity at EP_2, EP_3 and EP_4 receptors. AGN 192024 had no affinity for TP receptors. This study reconfirmed the unique pharmacology of AGN 192024.

15. Cardiovascular effects of AGN 192024. Report #: BIO-95-085, Vol. 12, Page 180

The purpose of this study was to determine the cardiovascular effects of AGN 192024 in anesthetized Sprague Dawley rats. The compound was administered intravenously at 10-1000 µg/kg. AGN 192024 caused a small and transient increase in mean arterial blood pressure at 100 µg/kg (10%) and 1 mg/kg (12%). At 1 mg/kg, AGN 192024 decreased heart rate up to 8% for up to 5 min. In summary, AGN 192024, at very high doses, could affect cardiovascular system in rats.

16. Effect of AGN 192024 and AGN 192151 on spontaneous motor activity. Report #: BIO-96-099, Vol. 12, Page 220

The purpose of this study is to investigate the effects of AGN 192024, which shared certain structural features with anandamide, an endogenous ligand for cannabinoid receptor, on locomotor activity in mice. In this study, AGN 192024 was administered intraperitoneally to mice. Anandamide (0.1, 1 and 10 mg/kg) was included in this study. The spontaneous motor activity was determined with an open field activity meter over a 45-min period. AGN 192024 did not affect spontaneous motor activity in an open field at intraperitoneal doses of 10 mg/kg. Anandamide at highest dose, 10 mg/kg, decreased spontaneous motor activity. The results suggested that AGN 192024 do not produce CNS depression or cannabinomimetic effects.

17. Two-day ocular irritation study of 0.03% AGN 192024-0.5% timolol combination probe formulations in New Zealand white rabbits. Report #: BIO-99-311, Vol. 13, Page 131

The purpose of this study was to determine the ocular discomfort in rabbits following topical applications (qid x 2 days) of isotonic and hypotonic formulations of 0.03% AGN 192024-0.5% timolol. The results indicated that the isotonic formulation of 0.03% AGN 192024-0.5% timolol was well-tolerated (< 10% occurrence of slight discomfort). The hypotonic formulations, both of 0.03% AGN 192024-0.5% timolol and its vehicle, produced 45.8% and 54.2% occurrence of slight-mild discomfort, respectively.

18. A one day ocular efficacy and safety study of 0.03% AGN 192024-0.5% timolol combination probe formulations administered twice daily in normotensive beagle dogs. Report #: BIO-99-319, Vol. 14, Page 055

The purpose of this study was to determine the ocular efficacy and safety in normotensive beagle dogs following topical applications (bid x 1 days) of isotonic and hypotonic formulations of 0.03% AGN 192024-0.5% timolol. The results indicated that both isotonic formulation and hypotonic formulation of 0.03% AGN 192024-0.5% timolol were effective (IOP ↓ up to 5.1 mmHg for hypotonic formulation and 5.7 mmHg for isotonic formulation) and well-tolerated with no irritation or discomfort observed.

19. In vitro pharmacology of [redacted] (AGN 197318). Report #: BIO-00-330, Vol. 14, Page 205

[redacted] was identified as a synthetic impurity in batches of AGN 192024 during the manufacturing process. The purpose of this study was to determine the activity of [redacted] at various prostanoid receptors and compare its potency with that of AGN 192024. The results showed that [redacted] was a weak agonist in a preparation with FP/hypotensive lipid receptors which was predictive for ocular hypotensive activity in humans, monkeys and dogs. Its potency was approximately 24-fold less than that of AGN 192024 (see table below). [redacted] had minimal interaction with other known prostanoid receptors examined. In conclusion, [redacted] was a weak agonist that exhibited selectivity for the prostanoid FP/hypotensive lipid receptors.

Potency of [redacted] and AGN 192024 in different prostanoid receptor preparations

Receptor	Preparation	[redacted]	AGN 192024 (EC_{50}, μM)
FP/Hypotensive lipid	Cat iris sphincter		0.038
$FP_{vasc}/EP_2/EP_2$	Rabbit jugular vein		4.625
EP_1	Guinea pig ileum		Inactive
TP_{vasc}	Rat thoracic aorta		Inactive
TP_{plat}	Human platelet		Inactive
DP/IP	Human platelet		Inactive

20. In vitro pharmacology of [redacted] Report #: BIO-00-333, Vol. 14, Page 231

[redacted] was identified as a synthetic impurity and a degradant in batches of AGN 192024 during the manufacturing process. The purpose of this study was to determine the activity of [redacted] at various prostanoid receptors and compare its potency with that of AGN 192024. The results showed that [redacted] was a moderate agonist in a cat isolated iris sphincter preparation with FP/hypotensive lipid receptors which was predictive for ocular hypotensive activity in humans, monkeys and dogs. Its potency was approximately 11-fold less than that of AGN 192024 (see table below). Both AGN 192024 [redacted] produced weak vasorelaxant responses in the histamine-precontracted rabbit isolated jugular vein, an FP_{vasc}, EP_2 and EP_4 preparation. In conclusion, [redacted] was a moderate agonist that exhibited selectivity for the prostanoid FP/hypotensive lipid receptors. [redacted] had minimal interaction with other known prostanoid receptors.

Potency of [redacted] l AGN 192024 in different prostanoid receptor preparations

Receptor	Preparation	[redacted]	AGN 192024 (EC_{50}, μM)
FP/Hypotensive lipid	Cat iris sphincter		0.038
$FP_{vas}/EP_4/EP_2$	Rabbit jugular vein		4.625
TP_{vas}	Rat thoracic aorta		Inactive

[redacted]

22. The effects of AGN 192024 on the isolated uterus from rabbit, mouse, rat and human. Report #: BIO-98-273, Vol. 12, Page 298

AGN 192024, a $PGF_{2\alpha}$ analog, does not stimulate $PGF_{2\alpha}$-sensitive, classic FP receptor. The purpose of this study was to compare the effects of AGN 192024 and 17-phenyl trinor $PGF_{2\alpha}$ (a selective classical FP receptor agonist) on isolated uterine preparations from mouse, rat, rabbit and human.

AGN 192024 was only weakly active in contracting the uterus of the mouse, rat, nonpregnant human and pregnant human. The responses to AGN 192024, at the highest concentrations (10^{-5} M) did not reach the 50% contraction level in the mouse and rat uterus and did not reach the T/B ratio of 1 (the area of agonist-induced contraction vs. the intrinsic spontaneous background) in the human myometrium. AGN 192024 was a potent contractile agent in the rabbit uterus.

17-phenyl trinor $PGF_{2\alpha}$ potently contracted isolated uterine smooth muscle of the rat, mouse and rabbit (see table below). 17-phenyl trinor $PGF_{2\alpha}$ was also a potent contractile agent in the human isolated uterus and was more active in the nonpregnant than pregnant myometrium. In human volunteers, multiple, topical ophthalmic dosing of 0.03% AGN 192024 for up to 14 days revealed no detectable conversion of AGN 192024 to 17-phenyl trinor $PGF_{2\alpha}$.

Potency of Potency of 17-phenyl trinor $PGF_{2\alpha}$ and AGN 192024 in contraction of isolated uterus

Agonist	Mouse EC_{50} (nM)	Rat EC_{50} (nM)	Rabbit EC_{50} (nM)
17-phenyl trinor PGF2α	8.6±2.9	7.8±2	0.7±0.1
AGN 192024	> 10,000	> 10,000	21.7±14

In summary, AGN 192024 was pharmacologically unique and had an activity profile different from the classical FP agonists in the uterus of the mouse, rat and human. The contractile effects in rabbit uterus might be rabbit specific. AGN 192024 would be devoid of uterotonic activity in human females after ocular dosing.

23. Comparison of the effects of AGN 192024, AGN 192151, natural prostaglandins and analogs on DNA synthesis. Report #: BIO-98-277, Vol. 13, Page 001

Stimulation of DNA synthesis in Swiss 3T3 cells was considered to be mediated by the prostanoid receptor with particular sensitivity to $PGF_{2\alpha}$ ($PGF_{2\alpha}$-sensitive receptor). In this study, prostanoid-induced DNA synthesis in Swiss 3T3 cells was characterized using prostanoid FP agonists, natural prostanoids, selective prostanoid DP, EP and TP agonists, and AGN 192024 and AGN 192151 to confirm that prostanoid-induced mitogenesis in Swiss 3T3 cells was an FP receptor-mediated event. AGN 192024 is a $PGF_{2\alpha}$ analog where the C1-carboxylic acid group has been replaced by an ethylamide and a phenyl group introduced at C17. AGN 192151 is a $PGF_{2\alpha}$ analog that has a primary amide substituted for the carboxylic acid group at C1 and a methyl ether group at C15. Both AGN 192024 and AGN 192151 did not stimulate the $PGF_{2\alpha}$-sensitive, classical FP receptor and were described by the sponsor as hypotensive lipids.

The results (see table below) showed that C1-acidic $PGF_{2\alpha}$ analogs had mitogenic activity while the C1-modified analogs, AGN 192024 and AGN 192151, were inactive as mitogens in the cultured Swiss 3T3 cells. The study results supported the presence of a $PGF_{2\alpha}$-sensitive receptor associated with mitogenesis in Swiss 3T3 cells and indicated that this event was prostanoid FP receptor-mediated. The findings also supported the pharmacological separation between C1-carboxylic acid $PGF_{2\alpha}$ analogs and ocular hypotensive lipids.

Stimulation of DNA synthesis in cultured Swiss 3T3 cells by different compounds

Compounds	Receptor type	DNA synthesis EC_{50} (nM)
$PGF_{2\alpha}$	FP	88.7±21.5
Fluprostenol	FP	17.9±6.7
17-phenyl $PGF_{2\alpha}$	FP	40.6±24.3
Latanoprost-free acid	FP	272.5±82.8
PGD_2	DP	> 10000
PGE_2	EP	> 10000
U46619	TP	Not active
AGN 192024	Unknown	Not active
AGN 192151	Unknown	>> 10000

24. Regional differences in response to AGN 192024 in the cat lung parenchyma. Report #: BIO-99-298, Vol. 13, Page 024

The purpose of this study was to determine the potential regional variations in responsiveness to AGN 192024, the FP receptor agonist 17-phenyl- $PGF_{2\alpha}$, the TP receptor agonist I-BOP in isolated cat lung parenchyma. The results (see table below) showed that there were no regional differences for 17-phenyl- $PGF_{2\alpha}$ and I-BOP. AGN 192024 was 10-fold more potent in preparations obtained from the periphery of the cat lung parenchyma than in preparations obtained from the adjacent, more medial region. These results supported the concept that AGN 192024 interacted with a unique target receptor.

Effects of different compounds on contraction of peripheral and adjacent, more medial regions of the cat lung parenchyma

	EC_{50} (nM)			
Compound	**AGN 192024 (Test 1)**	**AGN 192024 (Test 2)**	**17-phenyl trinor $PGF_{2\alpha}$**	**I-BOP**
Peripheral	47	55	22	0.3
Medial	419	359	36	0.39

25. AGN 192024 pretreatment does not antagonize the Ca^{2+} signal response to prostaglandin $F_{2\alpha}$ in mouse and human fibroblast. Report #: BIO-99-299, Vol. 13, Page 040

The purpose of this study was to determine the effects of AGN 192024 at the FP receptor in Swiss 3T3 cells and human CRL 1497 cells by measuring the transient Ca^{2+} concentration changes. Under stimulation of AGN 192024, no measurable Ca^{2+} transient responses were apparent until a 10^{-6} M concentration was exceeded. Pretreatment of cells with 10^{-6}M AGN 192024 did not alter the Ca^{2+} signal response to graded doses of $PGF_{2\alpha}$. This finding further supported that AGN 192024 did not elicit its effects by stimulating the FP receptor, but appeared to interact with a unique population of target receptor.

26. The effects of AGN 192024 on arteriolar diameter in the microvasculature associated with human retinal tissues grafted into the hamster creek pouch membrane. Report #: BIO-99-307, Vol. 13, Page 054

The purpose of this study was to determine the vasomotor activity of AGN 192024 in the microvasculature associated with human retinal tissues grafted into the hamster cheek pouch membrane. Localized topical microsuffusion of AGN 192024 over a broad range of concentrations (10^{-10} M to 10^{-5} M) did not induce any significant changes in retinal microvessel caliber relative to baseline resting diameter.

27. Comparison of the effects of AGN 192024, AGN 192151, and prostanoid FP agonists on mouse isolated ileum. Report #: BIO-99-309, Vol. 13, Page 118

The purpose of this study was to determine the effects of FP agonists with AGN 192024 and AGN 192151 at the prostanoid FP receptor. $PGF_{2\alpha}$ and fluprostenol (a potent and selective FP receptor agonist) produced concentration-related contractions in the mouse ileum with EC_{50} of 193 nM and 48 nM, respectively. AGN 192024 and AGN 192151 produced weak contractile responses over a 1 nM to 1 µM concentration range. At 10 µM, the ileal responses induced by AGN 192024 and AGN 192151 were 16% and 33% of the reference $PGF_{2\alpha}$ contraction at 10 µM. The EC_{50} for both AGN 192024 and AGN 192151 was > 10000 nM. The results indicated that AGN 192024 and AGN 192151 did not appear to interact with the FP receptor in the mouse ileum.

28. Effects of serotonin (5-HT), AGN 192024, AGN 192151, and prostanoid FP agonists on human isolated umbilical artery. Report #: BIO-99-312, Vol. 13, Page 138

The purpose of this study was to determine the effects of $PGF_{2\alpha}$, latanoprost-free acid, fluprostenol, AGN 192024 and AGN 192151 on contraction of the human isolated umbilical artery. Serotonin was taken as a positive control in this study. The results are summarized in the table below. Two classical FP receptor agonists, $PGF_{2\alpha}$ and latanoprost-free acid, produced weak

contractile responses, which was significantly antagonized by a TP antagonist SQ 29548 (1 μM), indicating TP receptors were involved in the responses to $PGF_{2\alpha}$ and latanoprost-free acid. Fluprostenol, a potent and selective FP receptor agonist, and AGN 192024 and AGN 192151 showed weak contractile responses at concentration of 10 μM. At this high concentration, the effects of these agonists were not likely to be specific for the FP receptor. In conclusion, FP receptors were minimally involved in the contraction of human isolated umbilical arterial ring preparations. $PGF_{2\alpha}$ and latanoprost-free acid had weak contractile activities that were mediated by human vascular TP receptors.

Pharmacological activities of different compounds in human isolated umbilical artery

Compound	C1-moiety	EC_{50} (nM)	EC_{50} (nM) under 1 μM of SQ 29548
5-HT	N/A	108	87
$PGF_{2\alpha}$	Acid	4900	> 10000
Fluprostenol	Acid	> 10000	> 10000
Latanoprost-free acid	Acid	8710	> 10000
AGN 192024	Ethylamide	> 10000	Not tested
AGN 192151	amide	> 10000	Not tested

29. Effect of AGN 192024 on intracellular [Ca^{2+}] in HEL cells. Report #: BIO-99-314, Vol. 13, Page 209

The purpose of this study was to determine the effects of AGN 192024 on human erythroleukemia (HEL) cells, a cell line that constitutively expresses prostanoid EP_1 and TP receptors. AGN 192024 produced no Ca^{2+} signal, indicating minimal interaction with human EP_1 and TP receptors. In contrast, PGE_2 and thromboxane mimetic I-BOP, used as positive controls, caused dose-dependent increase in intracellular Ca^{2+} concentration.

30. Effects of AGN 192024 and latanoprost on iris color in cynomolgus monkeys after 1 year of topical treatment-Allergan Study TX97026. Report #: BIO-00-328, Vol. 14, Page 173

The purpose of this study was to determine the effects of AGN 192024 0.03% and 4 other receptor-selective prostaglandin analogs, including latanoprost 0.005% (FP receptor), on iris pigmentation following once daily ocular topical treatment for 1 year in cynomolgus monkeys. Slide images taken at the end of the 1-year treatment period were compared with images taken at base line for changes in iris color. The color changes were assessed in a masked fashion using an arbitrary scoring system with a range from –3 to +3 that allowed the observers to score an increase and a decrease in iris pigmentation. A slight darkening of the iris was noted in 2 untreated monkeys, which was considered as an age-related effect. Similar changes were also noted in the animals treated with AGN 192024, AGN 194042 (EP_1), AH 13205 (EP_2) and sulprostone (EP_3) in both treated and untreated eyes. These subtle changes were also considered as age-related and not treatment-related. Latanoprost induced a darkening of the iris in the treated eyes that was significantly more pronounced than the age-related effects observed in the other treatment groups. In conclusion, unlike AGN 192024, AGN 194042, AH 13205 and sulprostone, latanoprost 0.005% caused a significant increase in iris pigmentation in cynomolgus monkeys during 12 months of treatment.

31. Iris color and pigment changes in cynomolgus monkeys after 1 year of topical treatment with AGN 192024, AGN 192151 and latanoprost-TSI [redacted] Studies 007-004 and 007-005. Report #: BIO-00-329, Vol. 14, Page 186

The purpose of this study was to determine the effects of AGN 192024 0.01% and 0.1%, AGN 192151 0.01% and 0.1% and latanoprost 0.1% on iris pigmentation following twice daily ocular topical treatment for 1 year in cynomolgus monkeys. The effects on iris pigmentation were assessed histologically, ultrastructurally and by comparing slide images taken before and after the 1-year treatment period. The results are summarized in the table below. In the affected irides, increased melanin synthesis was evident in stromal melanocytes with increased level of melanosome maturation. The drug-related pigmentation was not reversible after a 3-month recovery period. In conclusion, the potential of long-term treatment with AGN 192024 to cause iris hyperpigmentation appeared to depend on the drug concentration employed.

Pigmentation scores for iris color changes in cynomolgus monkeys after 1-year topical treatment

Treatment	Vehicle	AGN 192151		AGN 192024		Latanoprost	Untreated
Concentration		0.01%	0.1%	0.01%	0.1%	0.1%	
Mean*	0.12±0.10	0.97±0.24	1.12±0.37	0.09±0.11	1.45±0.27	1.50±0.36	-0.22±0.21
N	10	10	9	10	10	6	6

Score 0: no color difference; Score 1: subtle change in pigmentation; Score 2: moderate, more pronounced change in pigmentation; Score 3: severe change in pigmentation

32. Comparison of vasorelaxation of the rabbit jugular vein by AGN 192024 and AGN 191835 in the presence or absence of indomethacin. Report #: BIO-00-331, Vol. 14, Page 220

The purpose of these studies was to determine the effects of indomethacin, a cyclooxygenase inhibitor, on vasorelaxation produced by AGN 192024 and AGN 191835 (latanoprost free acid) in isolated, endothelium-intact, histamine-precontracted jugular vein preparations of the rabbit. Between tissues tested in the presence or absence of 1 μM indomethacin, the vasorelaxation response to both compounds at each concentration was not significantly different. The vasorelaxation effects were mediated by FP_{vasc} receptors located in the vascular endothelium of the rabbit jugular vein since the effects were lost following the removal of the endothelial cells.

Effects of AGN 192024 and AGN 191835 on vasorelaxation of rabbit isolated precontracted jugular vein preparations

Receptor	Preparation	Indomethacin	EC50 (nM) AGN 192024	EC50 (nM) AGN 191835
FP_{vasc}	Endothelium-intact	1 μM	2432±742	34.7
FP_{vasc}	Endothelium-intact	0	3519±1847	4.6±1.6
EP_2 and EP_4	Endothelium-denuded	1 μM	inactive	Inactive

Pharmacokinetics:

Ocular ADME

1. Ocular absorption and tissue distribution of ^{3}H-AGN 192024 in rabbits after a single ophthalmic administration of 0.1% ^{3}H-AGN 192024 ophthalmic formulation. Report #: PK-96-014, Vol. 40, Page 135

The objective of this study is to determine the ocular absorption and distribution of AGN-192024 and its metabolites following a single instillation of the ophthalmic solution into rabbit eyes. The concentration of ^{3}H-AGN 192024 was measured by liquid scintillation counting. For

each group, 3 rabbits were treated bilaterally and 1 rabbit was treated unilaterally (left eye only). Ocular tissues and blood samples were collected at 0.5, 1, 2, 4, 6 and 8 hours post-dose. AGN 192024 and its metabolites in selected tissues were analyzed by reversed phase [redacted] The results showed that AGN 192024 was rapidly absorbed and distributed into the rabbit eyes. While the concentrations in intraocular tissues were lower than those of the extraocular tissues, the drug levels in iris and ciliary body were the highest among all intraocular tissues. The concentrations of total radioactivity in the contralateral untreated eyes and systemic blood and plasma were very low. Six unknown metabolites of AGN 192024 (referred to as RP-I to RP-VI) were detected in plasma. Several metabolites (referred to as RE-I to RE-VI) were also found in the ocular tissues. Thirty min after administration of AGN 192024, the concentrations of unchanged ^{3}H-AGN 192024 in all ocular tissues accounted for less than 50% of the total radioactivity. Therefore, the metabolism of AGN 192024 was rapid.

Total radioactivity in ocular tissues, blood and plasma after a single ocular administration of 0.1% ^{3}H-AGN 192024 into rabbit eyes.

Tissue	C_{max} (ng-eq/g or ml)	T_{max} (Hr)	AUC (0-8 Hr) (ng-eq•hr/g or ml)
Upper Conjunctiva	1330±290	0.5	1960±200
Lower Conjunctiva	1800±400	0.5	2030±270
Cornea	1470±350	0.5	3290±360
Upper Sclera	410±56	0.5	1020±100
Lower Sclera	546±145	0.5	1050±110
Aqueous humor	129±28	2.0	448±52
Iris	231±51	0.5	982±136
Ciliary Body	178±28	0.5	429±49
Lens	4.78±1.6	6.0	21.9±4
Vitreous Humor	4.89±1.1	0.5	23.4±3
Choroid-Retina	129±26	0.5	256±23
Blood	3.53±0.2	0.5	4.81±0.3
Plasma	6.28±0.6	0.5	10.9±0.5

2. A study to determine the ocular absorption and tissue distribution of ^{3}H-AGN 192151 and ^{3}H-AGN 192024 after multiple ocular doses to cynomolgus monkeys. Report #: PK-97-013, Vol. 40, Page 211.

The objective of this study is to determine the ocular absorption and distribution of 0.1% ^{3}H-AGN-192024 ophthalmic solution and 0.1% ^{3}H-AGN 192151 ophthalmic solution following repeated, twice daily ocular administration to male cynomolgus monkeys for 9-10 days. The samples were collected at the time points indicated below and were analyzed for ^{3}H activity by liquid scintillation counting.

Sample Collection Schedule

Group N[a]	Sample Collection Time			
	Timepoint	Plasma	tears	Eye Tissues
1-14	Days 1 and 7: 0.5 hr after evening dose and 0.5 hr before morning dose	X	X	
8-14	Day 10: 0.5 hr before final dose	X	X	
1-7	Day 11: 0.5 hr before final dose	X	X	
1, 7, 8, 14	0.5 hr after final dose	X		X
2, 9	2 hr after final dose	X		X
3, 10	4 hr after final dose	X		X
4, 11	6 hr after final dose	X		X
5, 12	8 hr after final dose	X		X
6, 13	24 hr after final dose	X		X

* Totally 18 doses for ^{3}H-AGN 192024 (Groups 8-14) or 20 doses for ^{3}H-AGN 192151 (Groups 1-7), both eyes (Groups 8-13, 1-6), left eye only (Groups 7 and 14)

The results showed that the highest C_{max} and AUC values occurred in the extraocular tissues for both compounds. The C_{max} and AUC data indicated that significant amounts of both compounds were absorbed and distributed into the intraocular tissues such as iris and ciliary body following multiple ophthalmic administration. Drug levels in systemic blood and plasma were low with C_{max} values at 2.358 and 3.228 ng-eq/ml, respectively. In ocular tissues and tears from the contralateral untreated eyes, the drug levels were very low due to limited systemic absorption.

Distributions of radioactivity in ocular tissues, blood and plasma following twice daily ocular doses of 0.1% ^{3}H-AGN192024 and 0.1% ^{3}H-AGN192151 to monkey eyes

	^{3}H-AGN192024			^{3}H-AGN192151		
Sample	Cmax (ng-eq/g)	Tmax (hr)	AUCt (ng-eq•hr/g)	Cmax (ng-eq/g)	Tmax (hr)	AUCt (ng-eq•hr/g)
Upper conjunctiva	6197.163	2	59007.36	7499.197	8	79780.37
Lower conjunctiva	5480.475	0.5	46824.14	6576.814	6	76781.24
Upper eyelid	11789.418	8	164645.46	11114.615	0.5	132400.75
Lower eyelid	30161.874	8	335066.58	29695.678	0.5	191421.56
Upper sclera	3848.479	8	55529.55	4091.595	0.5	25049.62
Lower sclera	8998.025	2	59797.16	3363.148	8	52655.06
Cornea	2952.608	0.5	29242.72	3290.067	6	37820.43
Iris	329.815	0.5	2343.64	450.242	2	4237.21
Ciliary body	1156.959	0.5	6329.26	859.325	0.5	5514.96
aqueous humor	18.731	6	167.07	281.676	2	895.49
Vitreous humor	3.829	8	74.39	7.397	2	102.60
Lens	30.893	2	211.69	43.476	6	400.23
Choroid/Retina	518.240	2	5088.39	459.954	0.5	4452.71
Optic nerve	683.924	2	11672.23	1107.468	0.5	9674.40
Blood	2.358	8	51.13	7.095	2	83.35
Plasma	3.228	8	65.07	13.855	2	117.73

3. Ocular pharmacokinetics and metabolism of ^{3}H-AGN 192024 in monkeys for TSI study 3-C52 titled "A study to determine the ocular absorption and tissue distribution of ^{3}H-AGN 192151 and ^{3}H-AGN 192024 after multiple ocular doses to cynomolgus monkeys". Report #: PK-97-032, Vol. 41, Page 001.

The purpose of this study was to examine the ocular pharmacokinetics of ^{3}H-AGN 192024 in monkeys following twice daily ocular topical administration at the concentration of 0.1% for 10 days. Blood and various ocular tissue samples were collected at 0.5, 2, 4, 6, 8 and 24 hr after the final dose. AGN 192024 and its metabolites were analyzed by reversed phase [redacted]

PK parameters in ocular tissues are summarized in the table below. AGN 192024 was rapidly absorbed and distributed well in the monkey eye. The concentrations matched those of the total radioactivity concentrations in Study PK-97-013. Significant concentrations of AGN 192024 remained in most ocular tissues 24 hr after the last dose because of the low metabolic rates. Although the highest concentrations were found in the eyelids, sclera, and conjunctivae, significant concentrations were detected in iris (309 ng/g) and ciliary body (1060 ng/g). In plasma, the intact AGN 192024 concentration at 0.5 hr post dose was 0.935 ng/ml and was unable to be detected by 2 hr post-dose, indicating very low systemic exposure following ocular dose. Two unknown minor metabolites (ME III and ME IV) were detected in most of the ocular

tissues at trace levels. AGN 191522, the C1-acid metabolite of AGN 192024, was found only in plasma, aqueous humor, iris and ciliary body.

PK parameters for ocular tissues following twice daily ocular doses of 0.1% ^{3}H-AGN192024

Tissue	Cmax (ng/g or ml)	Tmax (hr)	AUC (0-24 hr) (ng•hr/g or ml)
Upper eyelid	11800	8	162000
Lower eyelid	30200	8	332000
Upper conjunctiva	6050	2	57100
Lower conjunctiva	5350	0.5	44400
Cornea	2890	0.5	27500
Upper sclera	3780	8	53500
Lower sclera	8760	2	57200
Aqueous humor	13	6	102
Iris	309	0.5	1970
Ciliary body	1060	0.5	5730

4. A study to determine the ocular absorption and tissue distribution of [^{3}H]-AGN 192151 and [^{3}H]-AGN 192024 after a single ocular dose to cynomolgus monkeys. Report #: PK-97-036, Vol. 41, Page 019

The purpose of this study is to determine the ocular absorption and tissue distribution of ^{3}H-AGN 192151 and ^{3}H-AGN 192024 after a single ocular dose to cynomolgus monkeys. The animals received a single 35 µl ophthalmic instillation of 0.1% ^{3}H-AGN 192151 (Groups 1-7) or 0.1% ^{3}H-AGN 192024 (Groups 8-14). The eyes of the Group 15 monkey were dosed with saline. Blood, tear and eye tissue samples were collected at 0.5, 2, 4, 6, 8 and 24 hr after dosing and were analyzed for ^{3}H activity by liquid scintillation counting.

The pharmacokinetic parameters are listed on the following table. Higher concentrations of ^{3}H-AGN 192151 and ^{3}H-AGN 192024 were found in extraocular tissues (eyelid, conjunctiva and cornea) than in intraocular tissues. In Groups 7 and 14 (only left eyes were treated), the levels of radioactivity in the tissues of treated left eye were similar to those of bilaterally treated eyes. In the contralateral untreated eyes, the levels of radioactivity in ocular tissues and tears were very low, indicating that the total radioactivity in the undosed eyes was derived from systemic absorption. Throughout the observation period, the plasma and blood concentrations of ^{3}H-AGN 192151 and ^{3}H-AGN 192024 were at ng/ml range.

PK parameters for ocular tissues, blood and plasma following a single bilateral ocular administration of 0.1% ^{3}H-AGN 192151 and ^{3}H-AGN 192024 to monkeys.

	^{3}H-AGN 192151			^{3}H-AGN 192024		
Eye tissue	Mean Cmax (ng-eq/g)	Mean Tmax (hr)	Mean AUC (ng-eq•hr/g)	Mean Cmax (ng-eq/g)	Mean Tmax (hr)	Mean AUC (ng-eq•hr/g)
Upper conjunctiva	3941.252	0.5	20725.00	3121.170	0.5	21168.37
Lower conjunctiva	9400.950	4	35912.10	2616.988	2	33000.58
Upper eyelid	14942.750	0.5	75353.02	1922.784	0.5	26451.73
Lower eyelid	12105.500	4	76765.84	2187.234	8	32713.30
Upper sclera	743.327	4	9130.19	1031.601	0.5	5375.97
Lower Sclera	790.525	4	8850.77	1173.970	2	7346.51
Cornea	1862.932	0.5	10438.99	731.208	0.5	3340.08
Iris	1614.394	0.5	3254.57	531.957	2	2562.39
Ciliary body	318.691	0.5	1557.15	375.562	0.5	1649.79
Aqueous humor	193.880	0.5	795.56	46.986	0.5	159.42
vitreous humor	0.906	6	11.79	0.675	2	4.07
Choroid/retina	86.970	0.5	396.56	134.972	2	654.28
Blood	4.420	0.5	15.31	0.94	0.5	8.06
Plasma	6.370	0.5	20.03	1.28	0.5	10.52

5. Ocular pharmacokinetics and metabolism of ^{3}H-AGN 192024 in monkeys for TSI study titled "A study to determine the ocular absorption and tissue distribution of [^{3}H]-AGN 192151 and [^{3}H]-AGN 192024 after a single ocular dose to cynomolgus monkeys". Report #: PK-98-003, Vol. 41, Page 272

The purpose of this study is to determine the ocular absorption and tissue distribution of ^{3}H-AGN 192024 after a single ocular dose to cynomolgus monkeys. The animals received a single 35 µl ophthalmic instillation of 0.1% ^{3}H-AGN 192024. Ocular samples were collected at 0.5, 2, 4, 6, 8 and 24 hr after dosing and were analyzed by reversed phase [redacted]

AGN 192024 was absorbed and distributed well in monkey ocular tissues (see table below). The drug concentrations obtained in this study matched those of the total radioactivity concentrations in Study PK-97-036, indicating that intact ^{3}H-AGN 192024 represented most of the total radioactivity. The extraocular tissues showed higher AGN 192024 concentrations than those of the intraocular tissues. In plasma, the intact AGN 192024 concentration at 0.5 hr post dose was 0.347 ng/ml and was unable to be detected by 2 hr post-dose, indicating very low systemic exposure following ocular dose. AGN 192024 was only slightly metabolized in monkey ocular tissues. C1-acid metabolite and 3 unknown minor metabolites (ME III, ME IV and ME VI) were detected in most of the ocular tissues.

PK parameters for ocular tissues after a single bilateral ocular administration of ^{3}H-AGN 192024 to monkeys.

	^{3}H-AGN 192024		
Eye tissue	Mean Cmax (ng/g)	Mean Tmax (hr)	Mean AUC (ng•hr/g)
Upper conjunctiva	3060	0.5	20300
Lower conjunctiva	2570	2	32100
Upper eyelid	1890	0.5	25900
Lower eyelid	2160	8	20300
Upper sclera	1010	0.5	5170
Lower Sclera	1150	2	6850
Cornea	558	0.5	2610
Iris	445	2	1470
Ciliary body	352	0.5	1450
Aqueous humor	34.5	0.5	110

6. In vitro binding of ^{3}H-AGN 192024 to synthetic melanin. Report #: PK-99-045, Vol. 43, Page 214.

The purpose of this study is to determine the melanin binding of ^{3}H-AGN 192024 by ultracentrifugation method in vitro. The binding was measured in synthetic melanin suspensions (1 mg/ml) that was spiked with ^{3}H-AGN 192024 at 0.5, 1, 2, 5, 50 or 250 µM. Drug-melanin suspensions were incubated for 6 hr at 37 °C. Radioactivity was measured by liquid scintillation counting. The results showed that the percentage of ^{3}H-AGN 192024 bound to melanin after 6 hr incubation ranged from 10.7% to 27.0% over the AGN 192024 concentrations tested. The overall extent of binding was not dependent upon concentration, and the binding was reversible. In conclusion, AGN 192024 reversibly bound to synthetic melanin with a mean binding of approximately 20% at concentrations of 0.5-250 µM after 6 hr at 37 °C in vitro.

7. Autoradiography following repeat ocular instillation of ^{3}H-AGN 192024 to albino rabbits. Report #: PK-99-109, Vol. 43, Page 281

The purpose of this study is to determine the ocular distribution of ^{3}H-AGN 192024 following repeat ocular instillation of ^{3}H-AGN 192024 solution (0.03%, 35 µl, left eyes, qd) to male albino rabbits for 7 days. The animals were sacrificed at 0.25, 0.5, 1, 2 and 24 hr after the final dose, and the heads were removed for qualitative analysis of the tissue distribution of radioactivity by the technique of whole-body autography. The results showed that ^{3}H-AGN 192024-related material was mainly associated with the ocular surface (cornea and conjunctiva) and to a lesser extent with the aqueous humor during 2 hr after the final dose. At 24 hr after the final dose, concentration was declined such that radioactivity was associated only with the cornea.

Systemic ADME

1. In vitro permeability coefficients of a series of $PGF_{2\alpha}$ analogues using human corneal and scleral tissue. Report #: PK-93-078, Vol. 40, Page 001

The purpose of this in vitro study was to assess the corneal and scleral permeabilities of 7 $PGF_{2\alpha}$ analogues including AGN 192024 using human corneal and scleral tissues. The results showed that AGN 192024 was a good corneal and scleral penetrator with permeability coefficients of 3.24×10^{-6} cm/sec in cornea and 14.5×10^{-6} cm/sec in sclera.

2. Pharmacokinetics of AGN 192024 in Sprague-Dawley rats following single intravenous or single oral administration. Report #: PK-98-035, Vol. 41, Page 329

The purpose of this study was to determine the pharmacokinetics of AGN 192024 following a single iv or oral administration in rats. Sprague Dawley rats (6/sex/group) were dosed at 1 mg/kg iv or 4 mg/kg po. Blood samples were collected at pre-dose and 2, 5, 10, 15, 30, 60, 90, 120, 240, 360, 480 and 1440 min after iv dosing and at pre-dose and 5, 15, 30, 60, 90, 120, 240, 360, 480, 720 and 1440 min after po dosing. Blood concentrations of AGN 192024 and its C1-acid metabolite, AGN 191522, were measured with an [redacted]

The results are summarized in the table below. AGN 192024 was eliminated rapidly in rat blood following iv administration. The blood concentrations of AGN 191522 were much lower than those of AGN 192024. AGN 192024 had a moderate oral bioavailability in rats (28%) following a single oral administration.

PK parameters of AGN 192024 and AGN 191522 in rats after single iv or po administration

N = 6/sex	AGN 192024			AGN 191522		
Intravenous	♂	♀	♂+♀	♂	♀	♂+♀
Cmax (ng/ml)				30.5±18.0	12.0±3.9	21.3±15.7
Tmax (hr)				0.033±0	0.042±0.020	0.038±0.014
$AUC_{0-\infty}$ (ng-hr/ml)	96.9±34.6	119±20	108±29	4.23±1.47	3.57±1.13	3.90±.28
T1/2 (hr)	0.927±0.803	0.625±0.189	0.776±0.573	0.370±0.191	0.348±0.090	0.359±0.141
Oral						
Cmax (ng/ml)	159±148	112±91	135±120	10.0±6.9	3.35±2.01	6.88±6.07
Tmax (hr)	0.083±0	0.083±0	0.083±0	0.083±0	0.111±0.068	0.097±0.048
$AUC_{0-\infty}$ (ng-hr/ml)	148±191	99.9±44.7	122±127	9.72±14.0	2.40±1.91	6.06±10.20
T1/2 (hr)	7.53±.40	5.12±1.06	6.22±2.60	0.737±0.546	0.451±0.264	0.594±0.432

3. Pharmacokinetics of AGN 192024 in CD rats following single intravenous or single oral administration. Report #: PK-00-025, Vol. 46, Page 001

The purpose of this study was to determine the pharmacokinetics of AGN 192024 following a single iv or oral administration in rats. Sprague Dawley rats (6/sex/treatment) were dosed orally or intravenously at 0.3 mg/kg. Blood samples were collected at pre-dose and 2, 5, 10, 20, 40, 60, 120, 240 and 360 min after dosing. Blood concentrations of AGN 192024 and its C1-acid metabolite, AGN 191522, were measured with an [redacted]

The results are summarized in the table below. AGN 192024 was eliminated rapidly in rat blood following iv administration. AGN 192024 had a low oral bioavailability in rats (5.9-9.8%) following a single oral administration.

PK parameters of AGN 192024 and AGN 191522 in rats after single iv or po administration

N = 6/sex	AGN 192024			AGN 191522		
Intravenous	♂	♀	♂+♀	♂	♀	♂+♀
Cmax (ng/ml)	212±15	184±77	198±55	6.00±0.79	3.94±1.78	4.97±1.70
Tmax (hr)				0.042±0.020	0.033±0	0.038±0.014
$AUC_{0-\infty}$ (ng-hr/ml)	44.7±6.3	44.0±18.5	44.4±13.2	1.40±0.42	1.15±0.24	1.29±0.36
T1/2 (hr)	0.286±0.012	0.907±0.645	0.597±0.543	0.170±0.025	0.227±0.066	0.196±0.054
Oral						
Cmax (ng/ml)	2.80±3.04	4.38±4.95	3.59±4.00	BLQ	BLQ	BLQ
Tmax (hr)	0.089±0.043	0.103±0.053	0.0958±0.0467			
$AUC_{0-\infty}$ (ng-hr/ml)	3.00±0.79	4.17±2.68	3.58±1.98			
T1/2 (hr)	2.78±1.76	3.75±.13	3.26±3.07			

BLQ: 0.25 ng/ml

4. Intravenous pharmacokinetics and oral bioavailability of AGN 192024 in cynomolgus monkeys. Report #: PK-98-036, Vol. 41, Page 354

The purpose of this study was to determine the pharmacokinetics of AGN 192024 following a single iv or oral administration in monkeys. The animals (3/sex) were dosed at 1 mg/kg iv or 4 mg/kg po. Blood samples were collected at pre-dose and 2, 5, 10, 15, 30, 60, 90, 120, 240, 360, 480, 720 and 1440 min after iv dosing and at pre-dose and 10, 20, 40, 60, 90, 120, 240, 360, 480, 720 and 1440 min after po dosing. Blood concentrations of AGN 192024 and its C1-acid metabolite, AGN 191522, were measured with an [redacted]

The results are summarized in the table below. The blood concentrations of AGN 191522 were much lower than those of AGN 192024. AGN 192024 had a very low oral bioavailability in monkeys (2.6%) following a single oral administration.

PK parameters of AGN 192024 and AGN 191522 in monkeys after single iv or po administration

N = 3/sex	AGN 192024			AGN 191522		
Intravenous	♂	♀	♂+♀	♂	♀	♂+♀
Cmax (ng/ml)				4.56±1.92	18.7±24.0	11.6±17.0
Tmax (hr)				0.195±0.048	0.528±0.459	0.361±0.344
$AUC_{0-\infty}$ (ng-hr/ml)	417±7	419±128	418±81			
T1/2 (hr)	8.71±6.46	8.17±4.40	8.44±4.95			
Oral						
Cmax (ng/ml)	17.9±3.56	37.2±23.3	27.6±18.3	Below limit of quantitation (< 0.25 ng/ml)		
Tmax (hr)	0.222±0.096	0.333±0.289	0.278±0.202			
$AUC_{0-\infty}$ (ng-hr/ml)	43.9±18.5	41.5±6.83	42.7±12.5			
T1/2 (hr)	13.5±.17	7.28±7.96	10.4±8.39			

5. Pharmacokinetics of AGN 192024 in Swiss-Webster mice following single intravenous or single oral administration. Report #: PK-98-037, Vol. 42, Page 001

The purpose of this study was to determine the pharmacokinetics of AGN 192024 following a single iv or oral administration in mice. Swiss-Webster mice (4/sex/time point) were dosed at 1 mg/kg iv or 4 mg/kg po. Blood samples were collected at pre-dose and 2, 5, 10, 15, 30, 60, 90, 120, 240, 360, 480 and 1440 min after iv dosing and at pre-dose and 10, 20, 40, 60, 90, 120, 240, 360, 480, 720 and 1440 min after po dosing. Blood concentrations of AGN 192024 and its C1-acid metabolite, AGN 191522, were measured with an [redacted]

The results are summarized in the table below. AGN 192024 was eliminated rapidly in mouse blood following iv administration. The blood concentrations of AGN 191522 were much lower than those of AGN 192024. AGN 192024 had a moderate oral bioavailability in mice (20.6% in females and 58.7% in males) following a single oral administration.

PK parameters of AGN 192024 and AGN 191522 in mice after single iv or po administration

N = 4/sex/time point	AGN 192024			AGN 191522		
Intravenous	♂	♀	♂+♀	♂	♀	♂+♀
Cmax (ng/ml)				14.2±0.53	43.7±16.0	29.0±19.2
Tmax (hr)				0.333	0.333	0.333
$AUC_{0-\infty}$ (ng-hr/ml)	125	140	133	Not calculable		
T1/2 (hr)	0.817	0.432	0.544	Not calculable		
Oral						
Cmax (ng/ml)	707±266	160±233	403±384	114±128	BLQ (< 0.5 ng/ml)	62.9±100.9
Tmax (hr)	0.167	0.333	0.167	0.167		0.167
$AUC_{0-\infty}$ (ng-hr/ml)	348	125	240	Not calculable		
T1/2 (hr)	0.321	1.91	1.49	Not calculable		

6. Pharmacokinetics of AGN 192024 in CD-1 mice following single intravenous or single oral administration. Report #: PK-00-017, Vol. 45, Page 359

The purpose of this study was to determine the pharmacokinetics of AGN 192024 following a single iv or oral administration in mice. CD-1 mice (6/sex/time point) were dosed orally or intravenously at 1 mg/kg. Blood samples were collected at pre-dose and 2, 5, 10, 20, 40, 60, 120, 240, and 360 min after dosing. Blood concentrations of AGN 192024 and its C1-acid metabolite, AGN 191522, were measured with an [redacted]

The results are summarized in the table below. AGN 192024 was eliminated rapidly in mouse blood following iv administration. The blood concentrations of AGN 191522 were much lower than those of AGN 192024. AGN 192024 had a moderate oral bioavailability in mice (15.2% in females and 72.1% in males) following a single oral administration.

PK parameters of AGN 192024 and AGN 191522 in mice after single iv or po administration

N = 4/sex/time point	AGN 192024			AGN 191522		
Intravenous	♂	♀	♂+♀	♂	♀	♂+♀
Cmax (ng/ml)	502	1090	796	12.0±5.9	20.3±11.8	16.2±9.9
Tmax (min)				2	2	2
$AUC_{0-\infty}$ (ng-hr/ml)	79.1	107	92.0	Not calculable		
T1/2 (hr)	0.347	0.157	0.255	Not calculable		
Oral						
Cmax (ng/ml)	302±109	128±278	215±221	4.13±2.04	2.66±4.92	3.39±1.69
Tmax (min)	2	2	2	2	2	2
$AUC_{0-\infty}$ (ng-hr/ml)	60.6	17.8	39.7	Not calculable		
T1/2 (hr)	0.218	1.29	0.790	Not calculable		

7. ^{3}H-AGN 192024: Tissue distribution in the rat following a single intravenous administration. Report #: PK-98-050, Vol. 42, Page 023

The purpose of this study was to determine the systemic distribution of AGN 192024 in Sprague-Dawley rats following single intravenous administration of ^{3}H-AGN 192024 at 1 mg/kg. Three animals/sex/time point were sacrificed at 0.5, 2, 5, 8, 24, 48 and 168 hr after dosing and blood and tissue samples were collected. Radioactivity concentrations were determined using liquid scintillation counting. The results are summarized in the table below. Following the single iv dosing, ^{3}H-AGN 192024 was rapidly and widely distributed throughout the animal body. In most tissues the maximal tissue concentrations were reached within 0.5 hr. High concentrations of radioactivity were found in the GI tract, liver, kidney and urinary bladder. The rapid appearance of radioactivity in the liver and GI tract suggested that biliary excretion might play a role in the disposition of AGN 192024 in rats.

Concentrations of radioactivity in tissues following a single oral dose of ^{3}H-AGN 192024 (1 mg/kg) in rats

Tissue/organ	Males				Females			
(µg-eq/g)	0.5 hr	5 hr	24 hr	168 hr	0.5 hr	5 hr	24 hr	168 hr
Plasma	0.403	0.159	0.155	0.035	0.410	0.242	0.185	0.049
Adrenal glands	0.185	0.076	0.093	0.017	0.281	0.129	0.112	0.021
Brain	0.125	0.128	0.133	0.031	0.177	0.200	0.162	0.045
Eye	0.125	0.115	0.124	0.025	0.157	0.148	0.137	0.032
Heart	0.251	0.132	0.134	0.032	0.317	0.202	0.163	0.044
Kidney	2.03	0.249	0.176	0.038	2.06	0.347	0.213	0.050
Liver	2.49	0.249	0.160	0.033	2.78	0.339	0.172	0.044
Lung	0.295	0.138	0.136	0.032	0.348	0.207	0.164	0.044
Ovary					0.196	0.107	0.089	0.023
Pancreas	0.314	0.127	0.120	0.030	0.372	0.184	0.146	0.041
Testis	0.174	0.141	0.144	0.033				
Thyroid	0.158	0.077	0.091	0.017	0.210	0.088	0.081	0.026
Stomach wall	1.33	0.891	0.108	0.023	2.30	1.77	0.229	0.027
Small intestine wall	5.41	2.78	0.202	0.023	6.46	2.01	0.266	0.026
Large intestine wall	1.59	7.74	0.492	0.019	1.81	5.35	0.452	0.027

8. ^{3}H-AGN 192024: Tissue distribution of radioactivity in male rats following repeated intravenous administration. Report #: PK-99-030, Vol. 43, Page 063

The purpose of this study was to determine the systemic distribution of AGN 192024 in albino rats following daily intravenous administration of ^{3}H-AGN 192024 at 0.084 mg/kg for 21 days. Three animals/time point were sacrificed at 0.5 hr after the first dose, 24 hr after 7 daily doses, 24 hr after 14 daily doses, and 0.5, 5, 24, 72 and 168 hr after the final dose and blood and tissue samples were collected. Radioactivity concentrations were determined using liquid scintillation counting. Qualitative whole-body autoradiography was also carried out on 1 rat at 0.5, 24 and 168 hr after the final dose.

Radioactivity was rapidly distributed to all tissues (see table below). The highest concentrations of radioactivity were found in the GI tract, liver and kidneys. The concentrations of radioactivity were greatest in the rats sacrificed at either 30 min or 5 hr after the final dose. The concentrations in all tissues showed a steady decline at 24, 72 and 168 hr after the final dose.

Concentrations of radioactivity in tissues following repeat administration of ^{3}H-AGN 192024 (0.084 mg/kg) in rats

(µg-eq/g)	Day 1 + 0.5 hr	Day 7 + 24 hr	Day 14 + 24 hr	Day 21 + 5 hr	Day 21 + 5 hr	Day 21 + 168 hr
Whole blood	0.022	0.050	0.061	0.109	0.110	0.029
Plasma	0.025	0.054	0.062	0.126	0.120	0.031
Adrenal glands	0.020	0.033	0.027	0.054	0.057	0.015

(µg-eq/g)	Day 1 + 0.5 hr	Day 7 + 24 hr	Day 14 + 24 hr	Day 21 + 5 hr	Day 21 + 5 hr	Day 21 + 168 hr
Brain	0.013	0.048	0.057	0.102	0.108	0.029
Eye	0.012	0.036	0.041	0.064	0.084	0.023
Heart	0.007	0.014	0.025	0.052	0.041	0.023
Kidney	0.084	0.062	0.078	0.213	0.141	0.041
Liver	0.114	0.051	0.064	0.204	0.118	0.030
Lung	0.023	0.047	0.061	0.116	0.109	0.030
Stomach wall	0.103	0.045	0.060	0.291	0.187	0.024
Small intestine wall	0.919	0.050	0.067	1.064	0.343	0.025
Large intestine wall	0.206	0.092	0.084	0.306	1.613	0.024

The whole body autoradiography examination showed a similar distribution to that seen in the quantitative study. At 168 hr after the final dose, radioactivity was not detected in any tissue or organ.

9. ^{3}H-AGN 192024: Placental transfer and milk secretion in the rat following a single intravenous administration. Report #: PK-93-078, Vol. 40, Page 001

The purpose of this study was to determine the placental transfer of radioactivity in pregnant rats, and to determine the secretion of radioactivity into the milk of lactating rats following a single intravenous dose (1 mg/kg) of ^{3}H-AGN 192024 in pregnant (on day 18 of pregnancy) and lactating (1 week after parturition) Sprague Dawley rats. Tissue and plasma samples from 3 animals per time point were collected at 0.5, 2, 5 and 24 hr (pregnant rats) and 0.5, 1, 3, 6 and 24 hr (lactating rats) after dosing. Tissue and plasma samples were analyzed for total radioactivity by liquid scintillation counting.

In the assay conducted in the pregnant rats, radioactivity was distributed rapidly and maximum concentrations was reached in most tissues within 30 min with the highest levels observed in the liver (2.44 µg-eq/g) and kidney (1.52 µg-eq/g). Radioactivity was noted in amniotic fluid (Cmax: 0.304 µg-eq/ml) and fetus (Cmax: 0.286 µg-eq/g), indicating placental transfer of radioactivity.

Concentrations of radioactivity in tissues following iv administration of ^{3}H-AGN 192024 (1 mg/kg) to pregnant rats on day 18 of pregnancy (µg-eq/g)

Tissue/organ	Time			
	0.5 hr	2 hr	5 hr	24 hr
Amniotic fluid	0.162	0.269	0.304	0.274
Fetus	0.189	0.256	0.286	0.265
Kidney	1.52	0.515	0.408	0.280
Liver	2.44	0.647	0.409	0.245
Placenta	0.349	0.273	0.281	0.254
Plasma	0.407	0.264	0.283	0.235

The results from the lactating rat assay showed that the secretion of radioactivity from ^{3}H-AGN 192024 into milk occurred during 24 hr after dosing (see table below).

Concentrations of radioactivity in blood, plasma and milk in rats treated with ^{3}H-AGN 192024

	µg-eq ^{3}H-AGN 192024/g		
Collection time (hr)	BLOOD	PLASMA	MILK
0.5	0.301	0.398	0.644
1	0.173	0.222	0.505
3	0.149	0.174	0.252
6	0.134	0.150	0.165
24	0.097	0.097	0.091

10. In vitro protein binding of ^{3}H-AGN 192024 in mouse, rat, rabbit, monkey and human plasma and bovine and human serum albumin using [redacted] Report #: PK-98-126, Vol. 43, Page 033

The purpose of this study was to determine the extent of in vitro plasma protein binding of ^{3}H-AGN 192024 in mice, rats, rabbits, monkeys and humans by [redacted] and liquid scitillation counting. The in vitro binding to bovine and human serum albumin was also assessed. The results are summarized in the table below. AGN 192024 was modestly free in animal plasma proteins (28-37%) and slightly unbound to human plasma [redacted] The extent of binding did not change over plasma AGN 192024 concentrations tested (1-250 ng/ml). AGN 192024 was a poor ligand to bovine and human serum albumin.

Plasma protein binding of AGN 192024 in different species

	Percent unbound (%)					
Drug concentration (ng/ml)	1	2	5	10	250	Mean
Mouse	32.6±9.6	25.0±0.7	26.5±2.0	25.1±2.5	29.9±2.9	27.8±3.3
Rat	36.0±1.5	41.8±7.2	38.3±4.0	34.6±6.7	36.4±2.3	37.4±2.8
Rabbit	35.6±2.2	37.7±4.0	35.2±1.9	37.4±3.2	35.9±1.1	36.4±1.1
Monkey	35.6±4.3	34.2±4.6	38.7±4.2	35.2±1.2	40.0±4.2	36.7±2.5
4.5% bovine serum albumin	73.9±21.8	63.9±10.3	63.0±9.4	59.1±1.6	65.7±17.4	65.1±5.5

1. In vitro protein binding of ^{3}H-AGN 192024 in human plasma, serum albumin and α1-acid glycoprotein using [redacted] Report #: PK-99-121, Vol. 43, Page 381

[redacted]

Protein binding of AGN 192024 in human plasma, serum albumin and α1-acid glycoprotein

	Percent unbound (%)

[redacted]

12. Metabolism of 13 prostaglandin $F_{2\alpha}$ analogs by rabbit and monkey ocular tissues in vitro. Report #: PK-97-066, Vol. 41, Page 179

The purpose of this study was to examine the metabolism of 13 C1-modified analogs of $PGF_{2\alpha}$ including AGN 192024 after 18 hr incubation with ocular tissues of rabbit and monkey in vitro. The disappearance of parent drug and formation of the respective C1-acid metabolites in both the incubation media and tissues were measured using a reversed-phase [redacted] with UV detection. All the compounds tested were, to some extent, converted to their respective C1-acid metabolites by each of the ocular tissues (cornea, sclera, conjunctiva, iris-ciliary body, and choroid/retina) of both species. The metabolic conversion in rabbit ocular tissues was found to be faster than in monkey ocular tissues.

13. In vitro metabolism of AGN 192024 in dog liver and lung slices. Report #: PK-97-023, Vol. 40, Page 295

The purpose of this study was to examine the metabolism of AGN 192024 in dog liver and lung slices. Dog lung and liver slices were prepared and incubated with AGN 192024 at a concentration of 0.1 mg/1.7 ml for 18 hours. The metabolism of AGN 192024 in these samples were measured by reversed phase [redacted] The results showed that AGN 192024 was metabolized in dog liver and lung slices. The percent intact parent drug remaining after 18 hr incubation with dog lung and liver slices relative to control (drug incubated in buffer without tissue slices) was 79.5% and 16.8%, respectively. The metabolites detected in this study included AGN 191522 (C1-acid of AGN 192024), AGN 192024-glucuronide, monohydroxylated AGN192024, dihydroxylated AGN 192024, and β-oxidated AGN 191522.

14. In vitro metabolism of $PGF_{2\alpha}$, AGN 192151 and AGN 192024 in rat lung slices. Report #: PK-94-100, Vol. 40, Page 032

The purpose of this study was to examine the metabolism of $PGF_{2\alpha}$, AGN 192024 and AGN 192151 in rat lung slices after incubation for 24 hr. The samples were analyzed by reversed phase [redacted] The results showed that both AGN 192024 and AGN 192151 disappeared with time. Approximately 67% AGN 192151 and 23% AGN 192024 were metabolized after 24 hr incubation with lung slices. In conclusion, AGN 192024 and AGN 192151 were metabolized by rat lung slices, and AGN 192151 was metabolized faster than AGN 192024 in lung slices.

15. In vitro metabolism of AGN 192151 and AGN 192024 in rat liver slices. Report #: PK-94-103, Vol. 40, Page 049

The purpose of this study was to examine the metabolism of AGN 192024 and AGN 192151 in rat liver slices after incubation for 4, 8 and 24 hr at 37°C. The samples were analyzed by reversed phase [redacted] The results showed that approximately 20, 50 and 80% AGN 192024 and 65, 90 and 99% AGN 192151 were metabolized after 4, 8 and 24 hr incubation, respectively. The metabolism of AGN 192024 in rat liver slices was characterized by deamidation, hydroxylation, conjugation (with sulfate or glucuronic acid) and β-oxidation of the aliphatic chain.

16. In vitro metabolism of AGN 191045, AGN 192151 and AGN 192024 in human liver slices using high pressure liquid chromatography and mass spectrometry. Report #: PK-95-013, Vol. 40, Page 088

[redacted]

17. In vitro metabolism of ^{3}H-AGN 192024 in rat, monkey and human liver slices. Report #: PK-97-004, Vol. 40, Page 183

The purpose of this study was to examine the metabolism of AGN 192024 in rat, monkey and human liver slices after incubation for 18 hr at 37 °C. The samples were analyzed by [redacted] The results showed that approximately 89, 84, 58 and 77% AGN 192024 were metabolized after 18 hr incubation with rat, male monkey, female monkey and human liver, respectively. The liver metabolic profiles of AGN 192024 were comparable among the species tested. AGN 192024 was deamidated (conversion to C-1 acid) and glucuronidated in liver slices of all the species tested.

18. In vitro metabolism of AGN 192024 in liver microsomes of mouse, rat, rabbit, monkey and human. Report #: PK-99-047, Vol. 43, Page 229

The purpose of this study was to compare the metabolite profile of AGN 192024 in human liver microsomes with those of mouse, rat, monkey and rabbit. ^{3}H-AGN 192024 was incubated with the liver microsomes from different species at 37 °C for 10-60 min. The samples were analyzed by [redacted] The results showed that AGN 192024 was extensively metabolized by human liver microsomes to 3 major and several minor metabolites. The 3 major metabolites were tentatively identified by [redacted] as the hydroxylated analogs of AGN 192024. The metabolite profiles of AGN 192024 in liver microsomes from different species were very similar. The 3 major human metabolites were also noted in each of the animal species tested. The mouse liver microsomes metabolized AGN 192024 more rapidly and several additional metabolites were formed. Only rabbit liver microsomes metabolized AGN 192024 to form significant amount of AGN 191522, the C1-acid metabolite of Agn 192024. In conclusion, The metabolite profiles was similar across all 5 species examined.

19. Identification of human hepatic cytochromes P-450 involved in the metabolism of AGN 192024. Report #: PK-99-037, Vol. 43, Page 193

The purpose of this study was to determine the specific cytochrome P-450 isoforms responsible for the metabolism of AGN 192024 in vitro. ^{3}H-AGN 192024 was incubated with human liver microsomes at 37 °C for 5-10 min. Reversed phase [redacted] was used to quantify the metabolites of AGN 192024. AGN 192024 was extensively metabolized by human liver microsomes to 3 major and 3 minor metabolites. The 3 major metabolites were tentatively identified by [redacted] as the hydroxylated analogs of AGN 192024. The formation rate of the 3 major metabolites were correlated best with the activity of CYP3A4/5 isozyme. The involvement of CYP3A4/5 in AGN 192024 metabolism was further confirmed by inhibition of metabolite formation by troleandomycin, a specific mechanism-based inhibitor of CYP3A4/5, and by ketoconazole, a specific competitive inhibitor of CYP3A4/5. An assay with cDNA expressed CYP3A4 also confirmed the involvement of CYP3A4/5 in AGN 192024 metabolism. In conclusion, the metabolism of AGN 192024 to its 3 major oxidative metabolites in human liver microsomes appeared to be catalyzed by CYP3A4/5.

20. Effect of AGN 192024 treatment on hepatic drug metabolizing enzymes in rat and monkey. Report #: PK-99-100, Vol. 43, Page 245

The purpose of this study was to determine if daily iv injection of AGN 192024 to rats and monkeys produced any significant induction or inhibition of hepatic microsomal enzyme activity.

Male and female Sprague Dawley rats and cynomolgus monkeys were treated with AGN 192024 (1 mg/kg, iv) or vehicle for 1 month. The livers were collected from all animals. Microsomes were prepared by differential centrifugation to measure total microsomal proteins and specific microsomal P450 enzyme activities. The results showed that treatment with AGN 192024 did not produce any clinically significant induction and inhibition of the activities of any of the microsomal cytochrome P450 enzymes tested in Sprague Dawley rats and cynomolgus monkeys.

21. Stability of AGN 192024 in rat, dog, monkey and human blood and plasma up to 24 hours at various temperature. Report #: PK-97-005, Vol. 40, Page 198

The purpose of this study was to examine the stability of ^{3}H-AGN 192024 in blood and plasma of rat, dog, monkey and human after incubation at various temperatures [redacted] The stability was monitored over a 24 hr period by [redacted]

In rat and dog blood, ^{3}H-AGN 192024 was quantitatively converted to the C-1 acid of AGN 192024. In monkeys, 3 minor metabolites were detected. AGN 192024 was not metabolized in human blood.

In rats, both blood cell and plasma enzymes were involved in metabolizing AGN 192024. In monkeys and dogs, enzymes in the blood cells were mainly involved in metabolizing AGN 192024.

In rat and dog blood (also in rat plasma), the rate of metabolism increased with increasing temperature. AGN 192024 was almost completely metabolized within 3 and 24 hr incubation at 37 °C. Temperature had no effects on the metabolic conversion of AGN 192024 in dog and monkey plasma, and in monkey blood.

In conclusion, AGN 192024 was rapidly metabolized in rat and dog blood but not in monkey and human blood. Blood cells played a significant role in the metabolic conversion in the dog and rat.

22. Stability of AGN 192024 in mouse and rabbit blood and plasma during storage at 0 °C, 22 °C and 37 °C for 24 hr. Report #: PK-99-135, Vol. 44, Page 001

The purpose of this study was to examine the stability of ^{3}H-AGN 192024 in blood and plasma of the mouse and rabbit after incubation at various temperatures (4, 22 and 37 °C). The stability was monitored over a 24 hr period by [redacted] In both mouse and rabbit blood and plasma, ^{3}H-AGN 192024 was quantitatively converted to the C-1 acid of AGN 192024. Metabolism increased with increasing temperature.

23. Metabolite profiles in maternal and fetal tissues following a single intravenous administration of ^{3}H-AGN 192024 to pregnant rats. Report #: PK-99-023, Vol. 43, Page 048

The purpose of this study was to determine the metabolite profiles in maternal and fetal tissues following a single iv administration of ^{3}H-AGN 192024 (2 mg/kg) to pregnant rats (Day 18 of pregnancy). Animals were sacrificed at 0.5, 2 and 5 hr after dosing and blood and tissue (liver, kidney, lung, uterus, placenta, ovary and fetus) samples were collected. Metabolite profiles were determined by reversed phase [redacted]

AGN 192024 was extensively metabolized in the pregnant rats. As many as 22 individual metabolite peaks were seen in the [redacted] profiles of the various tissues. AGN 192024 and AGN 191522 (C1-acid metabolite) were detected in every tissue analyzed. AGN 191522 was the major metabolite in the ovary and uterus (46.7% and 39.7%, respectively), while AGN 192024 was the major component seen in the fetal tissues (50.2%). The fraction of C1-acid in the fetus was quite small (6.25%).

24. Metabolite profiles in blood, urine, feces of rat, monkey and human following a single intravenous administration of ^{3}H-AGN 192024. Report #: PK-99-113, Vol. 43, Page 328

The purpose of this study was to determine the metabolite profiles in blood, urine and feces following a single iv administration of ^{3}H-AGN 192024 to rats (2 mg/kg), monkeys (1 mg/kg) and healthy humans [redacted] Metabolite profiles were also investigated in blood of mice and rabbits following a single iv dose at 1 mg/kg. Blood samples were collected at 5 min (monkeys), 10 min (humans) and 30 min (mice, rats and rabbits) after dosing. Urine samples were collected up to 4 hr (monkeys), 6 hr (humans) and 24 hr (rats) after dosing. Feces sample were collected up to 48 hr (monkeys), 120 hr (humans) and 24 hr (rats) after dosing. Samples were analyzed by reversed phase [redacted]

The results indicated that the blood, urinary and fecal metabolite profiles of rats, monkeys and humans were comparable. AGN 192024 was extensively metabolized in all species tested via deamination, hydroxylation, N-deethylation and glucuronidation. As many as 14 individual metabolite peaks were seen in the [redacted] profiles of the various species. AGN 192024 and AGN 191522 (C1-acid metabolite) were detected in the blood and feces of all species. AGN 192024 was the major circulating drug-related component in the rat, monkey, mouse and human, while AGN 191522 was the major component in rabbit blood. AGN 192024 was the largest fraction of radioactivity excreted in rat (8.5-14.2%) and monkey (19.4%) urine. Glucuronide conjugate of AGN 192024 was major urinary metabolite in human (26.4%) and monkey (11.2%) urine.

25. Urinary and fecal recovery of radioactivity after intravenous administration of ^{3}H-AGN 192024 to rats. Report #: PK-96-020, Vol. 40, Page 163

The purpose of this study is to determine the elimination pathways of AGN-192024 in rats following intravenous administration of ^{3}H-AGN 192024 (33 µCi/3 mg/3 ml/kg). Urine and feces samples were collected at 24 hr intervals up to 312 hours. All samples were analyzed for total radioactivity by scintillation counting. The results showed that intravenously administered ^{3}H-AGN 192024 dose was quantitatively recovered in urine and feces. The excretion of AGN 192024 was rapid because large amount of radioactivity ($\geq$ 80%) was recovered in first 24 hr. The rates of recovery in both genders were > 90%. Radioactivity in the feces after iv injection suggested that biliary excretion might play an important role in the elimination of ^{3}H-AGN 192024 and its metabolites. There was a sex difference in the urinary and fecal elimination of ^{3}H-AGN 192024 and its metabolites.

Percent of radioactivity recovered in the urine, feces, carcass, and cage wash following a 33 µCi/3mg/kg intravenous dose of ^{3}H-AGN 192024 to Sprague-Dawley rats

Matrix	Males	Females	Mean
Feces	69.0 ± 4.4	49.2 ± 7.7	59.1 ± 12.0
Urine	26.6 ± 2.3	42.4 ± 4.2	34.5 ± 8.9
Carcass	1.87 ± 0.42	1.27 ± 0.20	1.57 ± 0.44
Cage wash	0.11 ± 0.04	0.24 ± 0.09	0.17 ± 0.10
Rinse	0.26 ± 0.16	0.17 ± 0.05	0.21 ± 0.12
Total	97.9 ± 4.2	93.3 ± 4.0	95.6± 4.6

26. A mass balance and pharmacokinetics study of ^{3}H-AGN 192024 following single intravenous administration to cynomolgus monkeys. Report #: PK-97-026, Vol. 40, Page 308

The purpose of this study was to determine the excretion routes and rates of ^{3}H-AGN 192024 administered via intravenous bolus (1 mg/1ml/kg) to cynomolgus monkeys. Three animals per sex were administered ^{3}H-AGN 192024 via intravenous bolus once on Day 1. Blood, urine and feces samples were collected at the timepoints indicated below. ^{3}H activity of the collected samples was analyzed by liquid scintillation counting.

Sample Collection Schedule

Dose Route	Samples for radioanalysis (post-dose)		
	whole blood	Urine	Feces
IV	Predose, 5, 15, and 30 min; 1, 2, 4, 8, 12, 24, 48, 72, 96, 120, 144, 168, 192, 216, 240, 264, 288 and 312 hr	-24-0, 0-4, 4-8, 8-12, 12-24, 24, 24-48, 48-72, 72-96, 96-120, 120-144, 144-168, 168-192, 192-216, 216-240, 240-264, 264-288 and 288-312 hr	-24-0, 0-24, 24, 24-48, 48-72, 72-96, 96-120, 120-144, 144-168, 168-192, 192-216, 216-240, 240-264, 264-288 and 288-312 hr

The results are summarized in the tables below. The short elimination half-life indicated that AGN 192024 was rapidly cleared following intravenous administration. Urinary excretion was the primary route for elimination of AGN 192024. The majority of excretion occured within the first 4 hours. The total recovery was > 90%. No gender differences were observed in either pharmacokinetic or excretion profiles.

PK parameters of ANG 192024 equivalents following administration of ^{3}H-AGN 192024 to monkeys (1 mg/kg)

	Males (n=3)		Females (n=3)	
Parameter	Mean	SD	Mean	SD
	Whole blood			
Cmax (µg-eq/ml)	1.53	0.58	1.60	0.35
$AUC_{0-\infty}$ (µg-eq•hr/ml)	1.08	0.45	1.04	0.17
	Plasma			
Cmax (µg-eq/ml)	2.13	0.84	2.18	0.47
$AUC_{0-\infty}$ (µg-eq•hr/ml)	1.54	0.68	1.56	0.24
T1/2 (hr)	1.29	0.59	1.12	0.10

Total recovery of radioactivity at 312 hr following administration of ^{3}H-AGN 192024 to cynomolgus monkeys at 1 mg/kg

	Fraction of Dose (%)			
	Males		Females	
	Mean	SD	Mean	SD
Urine	63.94	10.18	58.09	3.04
Feces	24.19	6.93	31.25	5.39
Cage wash	0.16	0.28	0.44	0.45
Urine pan rinse	1.77	0.84	1.74	0.38
Total	90.06	3.57	91.53	2.38

The following PK studies were submitted but not reviewed:

1. Penetration of $PGF_{2\alpha}$, PGE_2, PGD_2 and AH 13250 through human cornea and sclera in vitro. Report #: PK-94-029, Vol. 40, Page 020

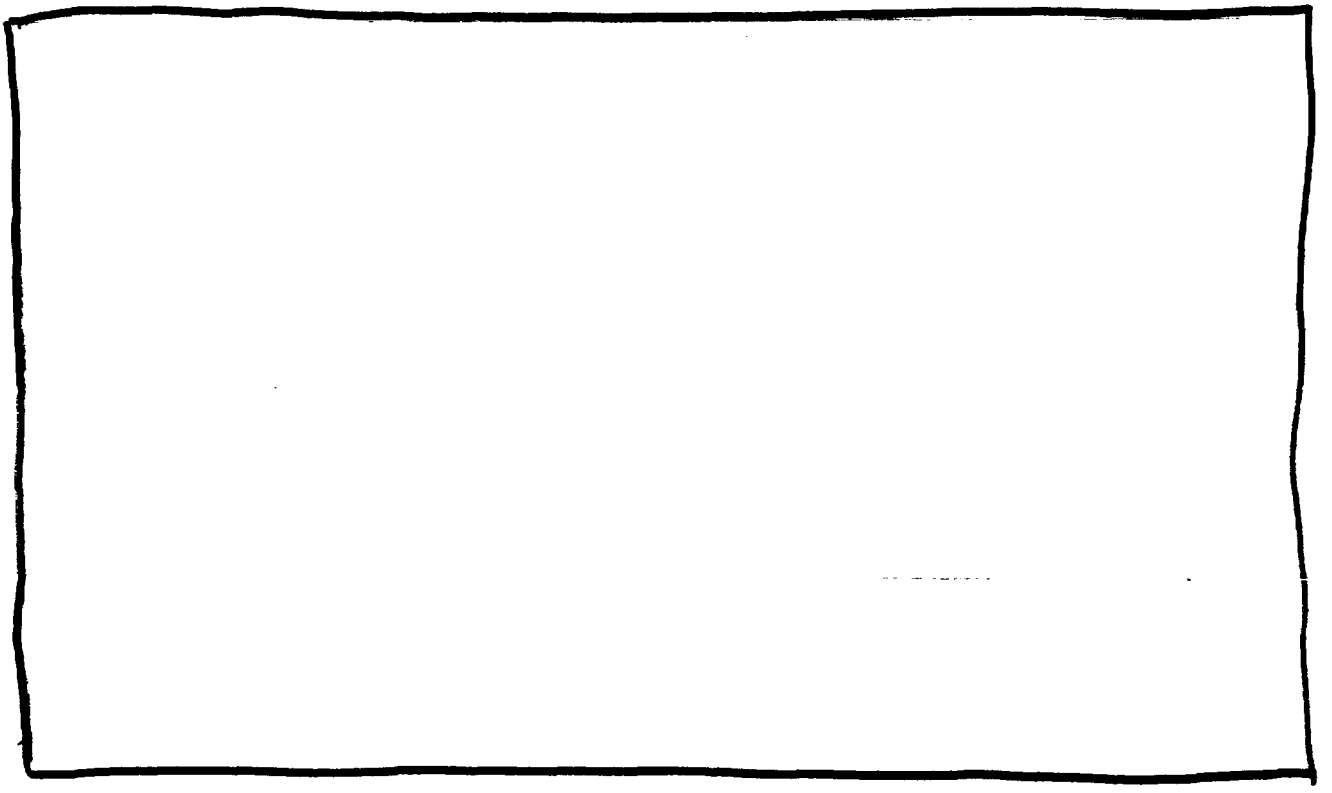
28. Bioanalytical method validation for the quantitation of AGN 192024 and AGN 191522 in rat blood using liquid chromatography-tandem mass spectrometry. Report #: PK-97-064, Vol. 41, Page 107

29. Bioanalytical method validation for the quantitating AGN 192024 and AGN 191522 in mouse blood using liquid chromatography-tandem mass spectrometry. Report #: PK-97-065, Vol. 41, Page 143

30. Bioanalytical method validation for the quantitation of AGN 192024 and AGN 191522 in rabbit blood using liquid chromatography-tandem mass spectrometry. Report #: PK-97-069, Vol. 41, Page 198

31. Bioanalytical method validation for the quantitation of AGN 192024 and AGN 191522 in monkey blood using liquid chromatography-tandem mass spectrometry. Report #: PK-97-070, Vol. 41, Page 236

32. Bioanalytical method validation for the quantitation of AGN 192024 and AGN 191522 in human blood using liquid chromatography-tandem mass spectrometry. Report #: PK-98-007, Vol. 41, Page 291



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Toxicology:

Systemic toxicity studies

1. AGN 192024: Acute single dose intraperitoneal injection safety study in Swiss-Webster mice. Vol. 15, Page 001

Study N^{o}: 1893-3137-7
Study Site: Allergan, Inc., 2525 Dupont Drive, Irvine, CA 92713-9534
Compound: AGN 192024 0.1% ophthalmic solution [redacted]
Route: Intraperitoneal
Dose Level: 96 mg/kg (96 ml/kg), single dose
Animal: Swiss-Webster mice, 3 mice/sex/group, 26-33 g
Study Initiation: June 6, 1995
GLP/QAU: Yes

The objective of this study was to establish a drug effect dose for AGN 192024 (0.1%) following a single ip injection in mice. The mice were dosed once intraperitoneally with AGN 192024 (0.1%) at 96 mg/kg. The day of dosing was designated as Day 1. The animals were observed for 7 days following treatment. Toxicity was assessed as shown below.

Toxicity assessment for Study 1893-3137-7

Parameter	Procedure
Clinical signs	Daily
Body weights	Days 1 and 7
Terminal observations	Gross pathological examinations were conducted in all animals on day 8.

Results:

Mortality and clinical signs: No mortality was observed and no clinical signs were noted during the 7-day study period.

Body weights: No effect was noted on body weights.

Terminal observations: No gross pathological changes were observed in any animal of any groups.

In summary, AGN 192024 when administered in mice as a single ip dose up to 96 mg/kg produced no local or systemic adverse effects.

2. AGN 192024: Acute single dose intravenous injection safety study in Sprague Dawley rats. Vol. 15, Page 037

Study N^{o}: 1012C-2968-59
Study Site: Allergan, Inc., 2525 Dupont Drive, Irvine, CA 92713-9534
Compound: AGN 192024 ophthalmic solution [redacted]

Route: Intravenous injection
Dose Level: 0.03, 0.3 and 3.0 mg/kg (3 ml/kg), single dose
Animal: Sprague Dawley rats, 6/sex/dose, ♂: 300-400g, ♀: 200-300 g
Study Initiation: February 9, 1995
GLP/QAU: Yes

The objective of this study was to determine the potential systemic toxic effects of AGN 192024 following a single iv injection in rats. The male and female Sprague Dawley rats were dosed once intravenously with AGN 192024 at 0.03, 0.3 and 3.0 mg/kg. The day of dosing was designated as Day 1. The animals were observed for 14 days following treatment. Toxicity was assessed as shown below.

Toxicity assessment for Study 1012C-2968-59

Parameter	Procedure
Clinical signs	At least once daily
Body weights	Weekly
Terminal observations	Gross pathological examinations were conducted in all animals on day 15.

Results:

Mortality and clinical signs: No mortality was observed and no signs of toxicity were noted during the observation period.

Body weights: No effect was noted on body weights.

Terminal observations: No gross pathological changes were observed in any animal of any groups.

In summary, AGN 192024 (0.1%) when administered in mice as a single ip dose up to 96 mg/kg produced no local or systemic adverse effects.

3. AGN 192024: toxicokinetics investigation following oral gavage administration to CD-1 mice for 2 weeks. Vol. 15, Page 067

Study N°: ALG 050/983734
Study Site: [redacted]
Compound: AGN 192024 0.2% ophthalmic solution (Lot #: 11268)
Route: Oral (by gavage)
Dose Level: 0.1, 0.3, 1 and 2 mg/kg/day for 2 weeks
Animal: Crl:CD-1 mice, 8-week old, 27-40 g for ♂ and 23-31 g for ♀, 18 mice/sex/group
Study Initiation: May 22, 1998
GLP/QAU: Yes

The objective of this study was to obtain PK information in mice orally treated with AGN 192024 for 2 weeks. The mice were dosed once daily with AGN 192024 at 0.1, 0.3, 1 or 2 mg/kg/day. The day of the 1st dosing was designated as Day 1. Toxicity was assessed as shown below.

Toxicity assessment for Study ALG 050/983734

Parameter	Procedure
Clinical signs	At least daily
Body weights	Weekly
Terminal observations	All animals were terminated on Day 14. No necropsy was performed.
PK	See Pharmacokinetics section.

Results:

Mortality and clinical signs: No treatment-related mortality and clinical signs were noted.

Body weights: There were no drug-related effects on body weights.

In summary, AGN 192024 when administered in CD-1 mice (oral, qd x 2 weeks) at the doses up to 2 mg/kg produced no clinical signs and mortalities.

4. AGN 192024: A 2-week oral toxicity study in Swiss-Webster mice. Vol. 15, Page 126

Study N^{o}: TX97003
Study Site: Allergan, Inc., 2525 Dupont Drive, Irvine, CA 92713-9534
Compound: AGN 192024 0.1% solution (Lot #: SJ031096-A03)
Route: Oral (by gavage)
Dose Level: 0, 10, 50 and 100 mg/kg/day x 14 days
Animal: Swiss-Webster mice, 6-7 weeks old, 17-24 g for ♂ and 17-23 g for ♀, 11 mice/sex/group (3/sex for main study and 8/sex for TK assay)
Study Initiation: April 3, 1997
GLP/QAU: No

The objective of this study was to determine the maximum tolerated dose and the systemic exposure of AGN 192024 in mice. The mice were orally (by gavage) dosed once daily with AGN 192024 (0.1%) at 10, 50 and 100 mg/kg for 14 days. The day of the 1st dosing was designated as Day 1. Toxicity was assessed as shown below.

Clinical Signs and Mortality – Prior to, immediately following dosing, and at least 2 times post-dosing
Body Weights – Weekly
Clinical Pathology – At study termination
TK Assay – See Pharmacokinetics section
Necropsy (Including Histopathology) – All animals received a complete necropsy examination. All tissues listed in the following table from all animals were processed and microscopically examined.

Adrenal	Cecum	Liver	Spleen
Brain	Colon	Jejunum	Thyroid
Epididymis	Testes	Lungs	Kidneys
Stomach	Heart	Ovaries	Duodenum
Ileum	Uterus and vagina		

Results:

Mortality and clinical signs: No treatment-related mortality was observed. No abnormal clinical signs were noted during the study period.

Body weights: No drug-related effect was noted on body weights.

Clinical pathology: No toxicologically significant, biologically relevant findings in hematology and clinical chemistry examinations were noted.

Gross necropsy: No drug-related gross pathological changes were observed in any animal of any group.

Histopathological evaluation: No toxicologically significant findings were observed.

In summary, Swiss Webster mice were treated orally with AGN 192024 at 10, 50 and 100 mg/kg/day for 2 weeks. No toxicity was observed. The dose of 100 mg/kg/day was determined as NOAEL in this study.

5. AGN 192024: A 2-week oral toxicity study in Swiss-Webster mice. Vol. 16, Page 001

Study Nº: TX97016
Study Site: Allergan, Inc., 2525 Dupont Drive, Irvine, CA 92713-9534
Study Purpose: To determine the maximum tolerated dose and the systemic exposure of AGN 192024 in mice.
Compound: AGN 192024 3.0% solution (Lot #: TT024-3%)
Vehicle: [redacted]
Route: Oral (by gavage)
Dose Level: 0, 250 and 500 mg/kg/day x 14 days
Animal: Swiss-Webster mice, 5-6 weeks old, 20-27 g for ♂ and 19-24 g for ♀, 3/sex/group for main study and 4/sex for TK assay
Study Initiation: May 21, 1997
GLP/QAU: No
Study Design: Mice (3/sex/group) were orally (by gavage) given 250 and 500 mg/kg AGN 192024 or vehicle control qd for 14 days. Toxicity was assessed as shown below.
Clinical Signs and Mortality – At least 4 times daily
Body Weights – Weekly
Clinical Pathology – At study termination.
TK Assay – See Pharmacokinetics section
Necropsy (Including Histopathology) – All animals received a complete necropsy examination. All tissues listed in the following table from all animals were processed and microscopically examined.

Adrenal	Cecum	Liver	Spleen
Brain	Colon	Jejunum	Thyroid
Epididymis	Testes	Lungs	Kidneys
Stomach	Heart	Ovaries	Duodenum
Ileum			

Results:

Mortality and clinical signs: No treatment-related mortality was observed. No abnormal clinical signs were noted during the study period.

Body weights: The body weight gain in 500 mg/kg group was slightly lower than in control group (♂: 2.1 g vs. control's 3.5 g; ♀: 1.2 g vs. control's 1.8 g). However, since the final body weights were similar between control and treated animals (♂: 27.1 g vs. control's 28.1 g; ♀: 22.9 g vs. control's 23.3 g), the changes in the body weight gain might not be toxicologically significant.

Clinical pathology: No toxicologically significant, biologically relevant findings in hematology and clinical chemistry examinations were noted.

Gross necropsy: No drug-related gross pathological changes were observed in any animal of any group.

Histopathological evaluation: No toxicologically significant findings were observed in both male and female animals receiving placebo and 250 mg/kg, and females receiving 500 mg/kg of AGN 192024. The only treatment-related lesion was an increase in the number of abnormal germ cells in the epididymides mostly in the caudal region in males of 500 mg/kg group. Since the caput epididymides were mostly normal, it was considered that the effects on the caudal epididymides was transient.

In summary, Swiss Webster mice were treated orally with AGN 192024 at 250 and 500 mg/kg/day for 2 weeks. The only treatment-related lesion was found in males of 500 mg/kg group evidenced by an minimal, transient increase in the number of abnormal germ cells in the epididymides mostly in the caudal region. No other toxicity was observed in this study. The dose of 250 mg/kg/day was determined as NOAEL in this study.

6. AGN 192024: A 2-week oral toxicity study in Sprague dawley rats. Vol. 18, Page 001

Study Nº: TX97002
Study Site: Allergan, Inc., 2525 Dupont Drive, Irvine, CA 92713-9534
Study Purpose: To determine the maximum tolerated dose and the systemic exposure of AGN 192024 in rats. The study was conducted to select doses for a 1-month oral toxicity study.
Compound: AGN 192024 1.0% solution (Lot #: SJ031096-A03)
Vehicle: [redacted]
Route: Oral (by gavage)
Dose Level: 0, 10, 50 and 100 mg/kg/day x 14 days (Dosing volume = 10, 1, 5 and 10 ml/kg)
Animal: Sprague Dawley rats, 6-7 weeks old, 152-174 g for ♂ and 142-156 g for ♀, 3/sex/group for main study and 2/sex for TK assay
Study Initiation: April 2, 1997
GLP/QAU: No
Study Design: Rats (3/sex/group) were orally (by gavage) given 10, 50 and 100 mg/kg AGN 192024 or vehicle control qd for 14 days. Toxicity was assessed as shown below.

Clinical Signs and Mortality – At least 4 times daily
Body Weights – Semiweekly
Clinical Pathology – At study termination on Day 14
TK Assay – See Pharmacokinetics section
Necropsy (Including Histopathology) – All animals received a complete necropsy examination. All tissues listed in the following table from all animals were processed and microscopically examined.

Brain	Duodenum	Adrenal	Testes and epididymides
Lungs	Jejunum	Kidneys	Thyroid
Heart	Ileum	Liver	
Spleen	Cecum	Ovaries	
Stomach	Colon	Uterus and vagina	

Results:

Mortality and clinical signs: No mortality was observed. No abnormal clinical signs were noted during the study period.

Body weights: No treatment-related differences in body weights were noted.

Clinical pathology: No toxicologically significant, biologically relevant findings in hematology and clinical chemistry examinations were noted.

Gross necropsy: No drug-related gross pathological changes were observed in any animal of any group.

Histopathological evaluation: Minimal degeneration of the right testis and the presence of abnormal germ cells in the ductal lumens of both epididymides were observed in 1 male at 100 mg/kg. Testicular degeneration (mild) with marked abnormal germ cells was also present in the left testis of one 50 mg/kg group male. Since these animals were 8-9 weeks old, the transition age between juvenile and sexually-matured rats, and degeneration occurred unilaterally in one testis only, it could not be ascertained that the lesion was due to AGN 192024 or simply a maturation process. No other toxicologically significant findings were observed.

In summary, Sprague dawley rats were treated orally with AGN 192024 at 10, 50 and 100 mg/kg/day for 2 weeks. The only possibly treatment-related lesion was found in one male each at 50 and 100 mg/kg groups evidenced by minimal to mild degeneration in 1 testis with abnormal germ cells in the epididymides. No toxicity was observed clinical observations, body weights and clinical pathology examinations in this study. The dose of 10 mg/kg/day was determined as NOAEL in this study.

7. AGN 192024: A 2-week oral toxicity study in Sprague Dawley rats. Vol. 18, Page 206

Study N^{o}: TX97015
Study Site: Allergan, Inc., 2525 Dupont Drive, Irvine, CA 92713-9534
Study Purpose: To determine the maximum tolerated dose and the systemic exposure of AGN 192024 in rats

Compound: AGN 192024 3.0% solution (Lot #: TT024-3%)
Vehicle: [redacted]
Route: Oral (by gavage)
Dose Level: 0, 250 and 500 mg/kg/day x 14 days (Dosing volume = 16.67, 8.33, 16.67 ml/kg)
Animal: Sprague Dawley rats, 5-6 weeks old, 120-140 g for ♂ and 110-130 g for ♀, 3/sex/group
Study Initiation: May 20, 1997
GLP/QAU: No
Study Design: Rats (3/sex/group) were orally (by gavage) given 250 and 500 mg/kg AGN 192024 or vehicle control qd for 14 days. Toxicity was assessed as shown below.

Clinical Signs and Mortality – At least 4 times daily
Body Weights – Semiweekly
Clinical Pathology – At study termination on Day 14
TK Assay – See Pharmacokinetics section
Necropsy (Including Histopathology) – All animals received a complete necropsy examination. All tissues listed in the following table from all animals were processed and microscopically examined.

Brain	Duodenum	Adrenal	Testes and epididymides
Lungs	Jejunum	Kidneys	Thyroid
Heart	Ileum	Liver	
Spleen	Cecum	Ovaries	
Stomach	Colon	Uterus and vagina	

Results:

Mortality and clinical signs: No mortality was observed. No abnormal clinical signs were noted during the study period.

Body weights: Decreased body weight gain was noted in all treated groups (see table below).

Body weight changes in animals treated with AGN 192024 (g)

	Males			Females		
Dosage (mg/kg)	**Vehicle**	**250**	**500**	**Vehicle**	**250**	**500**
Body weights						
Day 0	129±1.53	128±3.06	129±6.11	115±2.65	114±2.08	114±4.16
Day 14	236±7.09	194±10.44	177±16.77	167±4.58	139±9.85	139±7.37
% control		82.2	75		83.2	83.2
Body weight gain	107	66	48	52	25	25
% control		61.7	44.9		48.1	48.1

Clinical pathology: An increase in WBC counts was noted in male and female treated animals (see table below). BUN levels in the treated animals were also increased but creatinine levels were not increased. Without corresponding histopathological findings, the toxicological significance of these increases was not determined.

Clinical pathological findings in animals treated with AGN 192024

	Males			Females		
Dosage (mg/kg)	**Vehicle**	**250**	**500**	**Vehicle**	**250**	**500**
WBC ($10^3/\mu l$)	5.67±0.58	12.58±2.58	17.46±5.90	4.88±1.24	10.09±2.16	16.93±9.87
BUN (mg/dl)	14.33±2.52	24.33±3.79	24.33±3.51	21.33±2.08	29.67±5.86	28.00±4.24
Creatinine (mg/dl)	0.4±0.0	0.5±0.06	0.6±0.06	0.5±0.06	0.7±0.21	0.6±0.07

Gross necropsy: No drug-related gross pathological changes were observed in any animal of any group.

Histopathological evaluation: No drug-related, biologically relevant findings were noted.

In summary, Sprague Dawley rats were treated orally with AGN 192024 at 250 and 500 mg/kg/day for 2 weeks. Decreased body weight gain was noted in all treated groups. An increase in WBC counts and serum BUN levels were noted in male and female treated groups. Without corresponding histopathological findings, the toxicological significance of these changes was not determined. No NOAEL level was determined in this study.

8. AGN 192024: A 28-day oral toxicity study in Swiss-Webster mice. Vol. 16, Page 081

Study Nº: TX97034
Study Site: Allergan, Inc., 2525 Dupont Drive, Irvine, CA 92713-9534
Study Purpose: To determine the maximum tolerated dose and the systemic exposure of AGN 192024 in mice.
Compound: AGN 192024 0.2% solution (Lot #: 80597-02B)
Vehicle: [redacted]
Route: Oral (by gavage)
Dose Level: 0, 4, 8 and 16 mg/kg/day x 28 days
Animal: Swiss-Webster mice, 9-10 weeks old, 34-44 g for ♂ and 28-36 g for ♀, 10 mice/sex/group for main study and 9/sex for TK assay
Study Initiation: August 12, 1997
GLP/QAU: No
Study Design: Mice were orally (by gavage) given 4, 8 and 16 mg/kg AGN 192024 or vehicle control qd for 28 days. Toxicity was assessed as shown below.
Mortality – Daily
Clinical Signs and Mortality – At least 4 times daily
Body Weights – Weekly
Food Consumption – Weekly
Clinical Pathology – At study termination
TK Assay – See Pharmacokinetics section
Necropsy (Including Histopathology) – All animals were euthanized on Days 29 and 30. All animals received a complete necropsy examination. All tissues listed in the following table from the animals of control and high dose groups, and reproductive organs from all female animals were processed and microscopically examined.

Adrenal	Cecum	Mesenteric Lymph Nodes	Spleen
Brain	Colon	Ovaries	Thyroid
Epididymis	Rectum	Duodenum	Vagina and uterus
Stomach	Testes	Jejunum	Kidneys
Ileum	Heart	Lungs	Liver

Results:

Mortality and clinical signs: No mortality occurred. No abnormal clinical signs were noted during the study period.

Body weights: There were no toxicologically significant differences in body weights.

Food consumption: No drug-related, toxicologically significant changes in food consumption were noted.

Clinical pathology: No toxicologically significant, biologically relevant findings in hematology and clinical chemistry examinations were noted.

Gross necropsy: No drug-related gross pathological changes were observed in any animal of any group.

Histopathological evaluation: No toxicologically significant findings were observed in both male and female control and treated animals. Minimal mononuclear cell infiltration in the liver and kidneys, tubular degeneration and regeneration of the kidneys, hypoplasia of the testis with the associated reduced germ cell in the epididymides were noted in both control and treated animals (see table below). These changes were not considered toxicologically significant.

Histopathological findings in mice treated with AGN 192024

Dosage (mg/kg)	Males		Females			
	Control	16	Control	4	8	16
N	10	10	10	10	10	10
Liver: mononuclear cell infiltration, minimal	0	3	0			1
Kidneys: Tubular degeneration, minimal	0	2	1			0
mild	0	1	0			0
Kidney: Tubular regeneration, minimal	1	1	1			1
Kidney: Mononuclear cell infiltration, minimal	6	4	2			1
mild	1	1	0			1
Testis: hypoplasia, marked	1	0				
Epididymis: reduced germ cells, marked	1	0				

In summary, Swiss Webster mice were treated orally with AGN 192024 at 4, 8 and 16 mg/kg/day for 4 weeks. Toxicity evaluations included mortality, clinical signs, body weights, food consumption, clinical pathology, necropsy and histopathological examinations. No toxicity was observed in this study. The dose of 16 mg/kg/day was determined as NOAEL in this study.

9. AGN 192024: A 28-day oral toxicity study in Sprague Dawley rats. Vol. 19, Page 001

Study N^{o}: TX97035
Study Site: Allergan, Inc., 2525 Dupont Drive, Irvine, CA 92713-9534
Study Purpose: To determine the maximum tolerated dose and the systemic exposure of AGN 192024 in rats in order to select doses for a 3-month oral toxicity study.
Compound: AGN 192024 0.2% solution (Lot #: 80597-02A)
Vehicle: [redacted]
Route: Oral (by gavage)
Dose Level: 0, 4, 8 and 16 mg/kg/day x 28 days (Dosing volume = 8, 2, 4 and 8 ml/kg)

Animal: Sprague Dawley rats, 8-9 weeks old, 220-320 g for ♂ and 165-250 g for ♀, 8/sex/group for main study and 6/sex for TK assay
Study Initiation: August 21, 1997
GLP/QAU: No
Study Design: Rats were orally (by gavage) given 4, 8 and 16 mg/kg AGN 192024 or vehicle control qd for 28 days. Toxicity was assessed as shown below.
Mortality – Daily
Clinical Signs and Mortality – At least 4 times daily
Body Weights – Weekly
Food Consumption – Weekly
Clinical Pathology – At termination sacrifice on Days 29 and 30
TK Assay – See Pharmacokinetics section
Urinalysis – Urine samples were collected in Week 4 for urinalysis.
Necropsy (Including Histopathology) – All animals were euthanized on Days 29 and 30. All animals received a complete necropsy examination. All tissues listed in the following table from the animals of control and high dose groups, and reproductive organs and adrenal glands from all male animals were processed and microscopically examined.

Brain	Duodenum	Adrenal	Testes and epididymides
Lungs	Jejunum	Kidneys	Thyroid
Heart	Ileum	Liver	Mesenteric lymph node
Spleen	Cecum	Ovaries	
Stomach	Colon	Uterus and vagina	

Results:

Mortality and clinical signs: No mortality occurred. No abnormal clinical signs were noted during the study period.

Body weights: A decrease in body weight gain was seen in male animals (see table below). There were not significant differences in body weights among different groups. In females, no differences in body weights and body weight gain were noted. Therefore, the decrease in body weight gain in males might not be toxicologically significant.

Body weight changes in male rats treated with AGN 192024 (g)

Dosage (mg/kg/day)	Control	4	8	16
Week 0	273±12.7	272±12.2	273±11.4	273±11.0
Week 4	381±29.2	346±44.7	356±42.6	361±45.3
% control		90.8	93.4	94.8
Body weight gain	108.3	73.4	82.9	87.6
% control		67.8	76.5	80.9

Food consumption: No drug-related, toxicologically significant changes in food consumption were noted.

Clinical pathology: No toxicologically significant, biologically relevant findings in hematology and clinical chemistry examinations were noted.

Urinalysis: No drug-related changes in urinalysis were observed.

Gross necropsy: No drug-related gross pathological changes were observed in any animal of any group.

Histopathological evaluation: No toxicologically significant findings were observed in female animals. Bilateral degeneration of the testis and increased abnormal germ cells in the tubular lumen of the epididymis were present in 3 of 8 male animals treated with AGN 192024 at 16 mg/kg (see table below). These changes were considered treatment-related. A higher incidence of vacuolization of the cortical cells in the adrenal glands was seen in treated males. The significance of this adrenal change was not clear.

Histopathological findings in mice treated with AGN 192024

Dosage (mg/kg)	Males			
	Control	4	8	16
N	8	8	8	8
Adrenal gland: excessive vacuolization, cortex				
Minimal	2	5	3	5
Mild	0	0	0	1
Testis (right and left): degeneration, minimal	0	0	0	3
Epididymis (right and left): abnormal germ cells in tubular lumen, minimal	0	0	0	3

In summary, Sprague Dawley rats were treated orally with AGN 192024 at 4, 8 and 16 mg/kg/day for 4 weeks. No toxicity was observed in clinical observations, food consumption, clinical pathology, necropsy examinations. In male treated animals, a decrease in body weight gain was noted. Histopathological examinations in males at 16 mg/kg revealed bilateral degeneration of the testis and increased abnormal germ cells in the tubular lumen of the epididymis. The dose of 8 mg/kg/day was determined as NOAEL in this study.

10. AGN 192024: Toxicity study by oral gavage administration to CD-1 mice for 13 weeks followed by a 4-week recovery period. Vol. 17, Page 001

Study N^{o}: ALG 043/974324
Study Site: [redacted]
Compound: AGN192024 (0.2%, Lot#: 11220 and 11233)
Animal: Crl:CD-1 (ICR) BR mice (8 weeks old, 24-30 g for female; 30-39 g for male).
GLP: Yes

Dosing regimen for Study ALG 043/974324

			Number of mice (n/sex/group)			
Group	Dosage (mg/kg/day x 13 weeks, po)	Dose volume	Main	Recovery	TK	Total
1	Control (buffered saline)	8 ml/kg/day	10	5	♂3, ♀2	♂18, ♀17
2	4	2 ml/kg/day	10	5	9	24
3	8	4 ml/kg/day	10	5	9	24
4	16	8 ml/kg/day	10	5	9	24

The purpose of this study was to evaluate the toxicologic effects of AGN 192024 administered by gavage for 13 weeks followed by a 4-week recovery period in mice. The data would be used in selection of dosages for carcinogenicity study. Toxicity was assessed as shown below.

Toxicity assessment for Study ALG 043/974324

Parameter	Procedure
Mortality and clinical observations	Daily
Body weights	Weekly
Food consumption	Weekly
Ophthalmic examinations	Weeks 4 and 12 of treatment and Week 4 of recovery

Parameter	Procedure
Clinical pathology	Blood samples were collected from all main study mice after 13 weeks of treatment and 4 weeks of recovery.
Urinalysis	Urine samples were collected from all main study mice in Week 12 of treatment and Week 3 of recovery.
Gross pathology	At the end of the treatment or recovery periods, all of the main study mice were euthanized and necropsied.
Histopathology	The following tissues from all animals in Groups 1 and 4, and the ovary, vagina and thymus from all female animals were examined histologically: adrenals, aorta, brain, cecum, colon, duodenum, epididymis, eyes, femur with bone marrow, gall bladder, harderian gland, head, heart, ileum, jejunum, kidneys, lachrymal glands, liver, lungs, lymph nodes (mandibular and mesenteric), mammary area, esophagus, ovaries, pancreas, pituitary, prostate, rectum, salivary gland, sciatic nerve, seminal vesicles, skeletal muscle, spinal cord, spleen, sternum, stomach, testes, thymus, thyroids with parathyroids, tongue, trachea, urinary bladder, uterus with cervix, vagina and any gross lesions.
Organ weights	Organ weights were obtained for the following in each animal: brain, epididymides, heart, kidneys, liver, lungs, ovaries, prostate, pituitary, salivary glands, seminal vesicle, spleen, testes, thymus, uterus with cervix.

Results:

Mortality: No treatment-related death was noted. One female animal treated with 16 mg/kg/day of AGN192024 was found dead on Day 4, and one male animal treated with 4 mg/kg/day of AGN192024 was sacrificed on Day 35. The reasons for these deaths were unknown.

Clinical observations: No treatment-related clinical signs were noted.

Body weights: No toxicological significant differences in either body weights or body weight gains were noted.

Food consumption: There were no statistically significant differences in food consumption.

Ophthalmoscopy: No treatment-related effects were noted.

Clinical chemistry: No toxicologically significant differences were noted.

Hematology: The total number of WBC was decreased in several treated groups (see table below). Since no corresponding pathological findings were noted, and the degree of the decrease was not large, the changes may not be toxicologically significant.

Total WBC counting following treatment of AGN192024 in mice (10^9/l)

Time		Control	4 mg/kg/day	8 mg/kg/day	16 mg/kg/day
Week 13 (end of treatment)	♂	5.98	5.97	6.14	5.07
Week 17 (end of recovery)	♂	4.40	4.86	4.10	3.30
Week 13 (end of treatment)	♀	4.01	4.62	2.75	4.01
Week 17 (end of recovery)	♀	3.72	2.63	2.23	2.22

Urinalysis: No toxicologically meaningful events were noted.

Gross pathology: No treatment-related findings were noted.

Histopathology: an increased incidence of medullary lymphoid proliferation in the thymus for females at 8 or 16 mg/kg/day, and increased incidence of acute inflammatory cells in the superficial layers of the vagina for females at 16 mg/kg/day were observed (see table below). Thymus changes were fully recovered. The changes in the vagina were partially recovered during the 4-week recovery period. One female animal from high dose group showed extensive deposits of lymphoma throughout many organs. A pulmonary adenoma was detected in 1 male from 16 mg/kg/day after recovery period. No other toxicologically significant findings were observed.

Incidence of several histopathologic changes in mice after being treated with AGN192024

		males				Females			
Dosage (mg/kg/day)		0	4	8	16	0	4	8	16
Inflammatory cell in the superficial layer of vagina	Main					3/9	4/10	3/10	7/9
	Recovery					3/5	2/5	1/5	3/5
Thymus medullary lymphoid proliferation	Main	0/10	0/2	0/4	1/10	1/9	1/10	4/10	6/9
	Recovery	0/3	0/1	0/4	0/4	1/5	1/5	1/5	0/5

Organ weights: No toxicologically significant differences were noted.

[redacted]

Conclusion: Mice were treated with AGN192024 for 13 weeks followed by a 4-week recovery period. No treatment-related mortality, clinical signs, body weight and food consumption changes, and ophthalmoscopic findings were observed. Histopathology examinations showed increased incidence of medullary lymphoid proliferation in the thymus for females at 8 or 16 mg/kg/day, and increased incidence of acute inflammatory cells in the superficial layers of the vagina for females at 16 mg/kg/day. One female animal from high dose group showed extensive deposits of lymphoma throughout many organs. A pulmonary adenoma was detected in 1 male from 16 mg/kg/day after recovery period. No other toxicologically significant findings were noted. Plasma drug exposures were available in all of the treated groups.

11. AGN 192024: Toxicity study by oral gavage administration to CD rats for 13 weeks followed by a 4-week recovery period. Vol. 19, Page 171

Study N°: ALG 044/982455
Study Site: [redacted]
Compound: AGN192024 (0.2%, Lot#: 11246, purity = 99.8%)
Vehicle: [redacted]
Animal: Crl:CD BR rats (8 weeks old, 155-183 g for ♀; 203-242 g for ♂)
Study Initiation: February 18, 1998

GLP: Yes

Dosing regimen for Study ALG 044/982455

			Number of rats (n/sex/group)			
	Dosage (mg/kg/day x 13 weeks, po)	Dose volume	Main	Recovery	TK	Total
1	Control (buffered saline)	5 ml	10	5	3	18
2	0.1	0.5 ml	10	5	6	21
3	0.3	1.5 ml	10	5	6	21
4	1.0	5 ml	10	5	6	21

The purpose of this study was to evaluate toxicologic effects of AGN192024 administered by gavage for 13 weeks in rats. Toxicity was assessed as shown below.

Toxicity assessment for Study ALG 044/982455

Parameter	Procedure
Mortality and clinical observations	Daily
Body weights	Weekly
Food consumption	Weekly
Ophthalmic examinations	Weeks 4 and 12 of treatment and Week 4 of recovery. Indirect ophthalmoscopy and slit-lamp biomicroscopy examinations were performed on main and recovery animals.
Clinical pathology	Blood samples were collected from all main study rats in Week 13 of treatment and week 4 of recovery.
Urinalysis	Urine samples were collected from all main study rats in Week 12 of treatment and Week 3 of recovery.
Gross pathology	At the end of the treatment or recovery periods, all of the main study or recovery rats were euthanized and necropsied.
Histopathology	The following tissues from all animals in Groups 1 and 4, and the ovary from all animals were examined histologically: adrenals, aorta, brain, cecum, colon, duodenum, epididymis, eyes, femur with bone marrow, harderian gland, head, heart, ileum, jejunum, kidneys, lachrymal glands, liver, lungs, lymph nodes (mandibular and mesenteric), mammary area, esophagus, ovaries, pancreas, pituitary, prostate, rectum, salivary gland, sciatic nerve, seminal vesicles, skeletal muscle, spinal cord, spleen, sternum, stomach, testes, thymus, thyroids with parathyroids, tongue, trachea, urinary bladder, uterus with cervix, vagina and any gross lesions.
Organ weights	Organ weights were obtained for the following in each animal: adrenals, brain, epididymides, heart, kidneys, liver, lungs, ovaries, prostate, pituitary, salivary glands, seminal vesicle, spleen, testes, thymus, thyroid with parathyroids, uterus with cervix.

Results:

Mortality: No treatment-related death was noted.

Clinical observations: No treatment-related clinical signs were observed.

Body weights: No toxicologically significant differences in either body weights or body weight gain were noted.

Food consumption: There were no treatment-related effects on food consumption.

Ophthalmoscopy: No treatment-related changes were observed.

Clinical chemistry: No toxicologically significant differences were noted.

Hematology: No toxicologically significant differences were noted.

Urinalysis: No toxicologically meaningful events were noted.

Gross pathology: No treatment-related findings were noted.

Histopathology: Prominent corpora lutea (total of more than 70 for both ovaries) was found in 1 female at 0.3 mg/kg/day. An increase in numbers of vacuolated corpora lutea was noted in females receiving ≥ 0.3 mg/kg/day of AGN 192024, which were completely reversible after recovery period (see table below).

Incidence of several ovary changes in female rats after being treated with AGN192024

Dosage (mg/kg/day)	0	0.1	0.3	1.0
Terminal kill				
Number of pairs of ovaries examined	10	10	10	10
Cellular vacuolation in corpora lutea (total)	0/10	0/10	4/10	8/10
Minimal	0	0	4	3
Slight	0	0	0	5
Mean number of vacuolated corpora lutea	0	0	1.85	9.44
Mean total number of corpora lutea	41.22	38.32	47.82	49.55
Recovery kill				
Number of pairs of ovaries examined	5	5	5	5
Mean number of vacuolated corpora lutea	0	0	0	0
Mean total number of corpora lutea	29.31	39.10	44.65	45.43

Organ weights: ovary weight in treated animals was increased (see table below). No other toxicologically significant differences were noted.

Ovary weight in rats treated with AGN192024 for 13 weeks (mg)

Dosage (mg/kg/day)	0	0.1	0.3	1.0
Terminal kill	87.6	93.2	104.3	101.2
Recovery kill	87.3	103.7	96.6	109.1

[redacted]

Conclusion: Rats were treated orally with AGN192024 for 13 weeks followed by a 4-week recovery period. Treatment-related changes were characterized by vacuolated corpora lutea in animals treated with 0.3 and 1.0 mg/kg/day of AGN 192024, which was reversible after recovery period. Total number of corpora lutea was also slightly increased in these animals. Increased ovary weights were noted in treated females.

12. AGN 192024: Toxicity study by oral gavage administration to CD rats for 13 weeks followed by a 4-week recovery period. Vol. 20, Page 149

Study N^{o}: ALG 042/974323
Study Site: [redacted]
Compound: AGN192024 (0.2%, Lot#: 11220 and 11233, purity = 99.1% and 98.7%)
Vehicle: [redacted]
Animal: Crl:CD BR rats (8 weeks old, 169-220 g for ♀; 249-311 g for ♂)
Study Initiation: December 9, 1997
GLP: Yes

Dosing regimen for Study ALG 042/974323

			Number of rats (n/sex/group)			
Group	Dosage (mg/kg/day x 13 weeks, po)	Dose volume	Main	Recovery	TK	Total
1	Control (buffered saline)	8 ml	10	5	3	18
2	4	2 ml	10	5	6	21
3	8	4 ml	10	5	6	21
4	16	8 ml	10	5	6	21

The purpose of this study was to evaluate toxic effects of AGN 192024 administered by gavage for 13 weeks followed by a 4-week recovery period in rats. The day of the first treatment was designated as Day 0. Toxicity was assessed as shown below.

Toxicity assessment for Study ALG 042/974323

Parameter	Procedure
Mortality and clinical observations	At least once daily
Body weights	Weekly
Food consumption	Weekly
Ophthalmic examinations	Weeks 4 and 12 of treatment and Week 4 of recovery. Indirect ophthalmoscopy and slit-lamp biomicroscopy examinations were performed on main and recovery animals.
Clinical pathology	Blood samples were collected from all main study and recovery rats in Week 13 of treatment and week 4 of recovery.
Urinalysis	Urine samples were collected from all main study and recovery rats in Week 12 of treatment and Week 3 of recovery.
Gross pathology	At the end of the treatment or recovery periods, all of the main study or recovery rats were euthanized and necropsied.
Histopathology	The following tissues from all animals in Groups 1 and 4, and the ovary from all animals were examined histologically: adrenals, aorta, brain, cecum, colon, duodenum, epididymis, eyes, harderian gland, head, heart, ileum, jejunum, kidneys, lachrymal glands, liver, lungs, lymph nodes (mandibular and mesenteric), mammary area, esophagus, ovaries, pancreas, pituitary, prostate, rectum, salivary gland, sciatic nerve, seminal vesicles, skeletal muscle, spinal cord, spleen, sternum, stomach, testes, thymus, thyroids with parathyroids, tongue, trachea, urinary bladder, uterus with cervix, vagina and any gross lesions.
Organ weights	Organ weights were obtained for the following in each animal: adrenals, brain, epididymides, heart, kidneys, liver, lungs, ovaries, prostate, pituitary, salivary glands, seminal vesicle, spleen, testes, thymus, thyroid with parathyroids, uterus with cervix.
[redacted]	

Results:

Mortality: No treatment-related death was noted.

Clinical observations: No treatment-related clinical signs were observed.

Body weights: Average body gains are listed in the table below. All treated animal groups demonstrated a decrease in body weight gain. During the recovery period, there were no differences in body weight gains among different groups.

Body weight changes caused by AGN 192024 administered orally for 13 weeks (g)

	Males				Females			
Mean body Weights	Dosage (mg/kg/day)							
	Control	4	8	16	Control	4	8	16
Start of treatment	276	285	282	280	199	200	199	202
Week 13	517	509	482	464	292	278	277	279
% of control	100	98.5	93.2	89.7	100	95.2	94.9	95.5
Body weight gain	241	224	200	184	93	79	78	75
% of control	100	92.9	83	76.3	100	85	84	81

Food consumption: Animals receiving high dose showed a slight decrease in food consumption (see table), which was reversed during the recovery period.

Food consumption in rats treated with AGN 192024 by oral gavage for 13 weeks

	Males				Females			
Food Consumption (g/rat/week)	Group dosage (mg/kg/day)							
	Control	4	8	16	Control	4	8	16
Week 1	219	215	217	209	155	151	146	150
Week 1-13	2927	2896	2821	2839	2122	2032	2027	1984
% of control	100	99	96	97	100	96	96	93
Week R1-R4	863	920	914	997	686	626	611	651
% of control	100	107	106	116	100	91	89	95

Ophthalmoscopy: No treatment-related changes were observed.

Clinical chemistry: Treatment-related increases in GOT and GPT levels were observed in male treated rats, which returned to the control level after 4 weeks recovery period (see table below). These changes were not corroborated by histopathological changes. No other toxicologically significant differences were noted.

GOT and GPT activities in rats treated with AGN192024 by oral gavage for 13 weeks

	Males				Females			
mu/ml	Dosage (mg/kg/day)							
Week 13	Control	4	8	16	Control	4	8	16
GPT	34	59	106	155	32	31	36	32
GOT	63	94	151	255	56	60	60	63
Week 17								
GPT	36	29	28	29	49	30	30	20
GOT	61	54	51	59	71	48	52	46

Hematology: No toxicologically significant differences were noted.

Urinalysis: No toxicologically meaningful events were noted.

Gross pathology: No treatment-related findings were noted.

Histopathology: Prominent corpora lutea (total of more than 70 for both ovaries) as well as an increase in numbers of vacuolated corpora lutea was found in all treated groups. The increase in treated animals demonstrated a dose-dependent manner. These changes were partially reversible after 4 weeks recovery period.

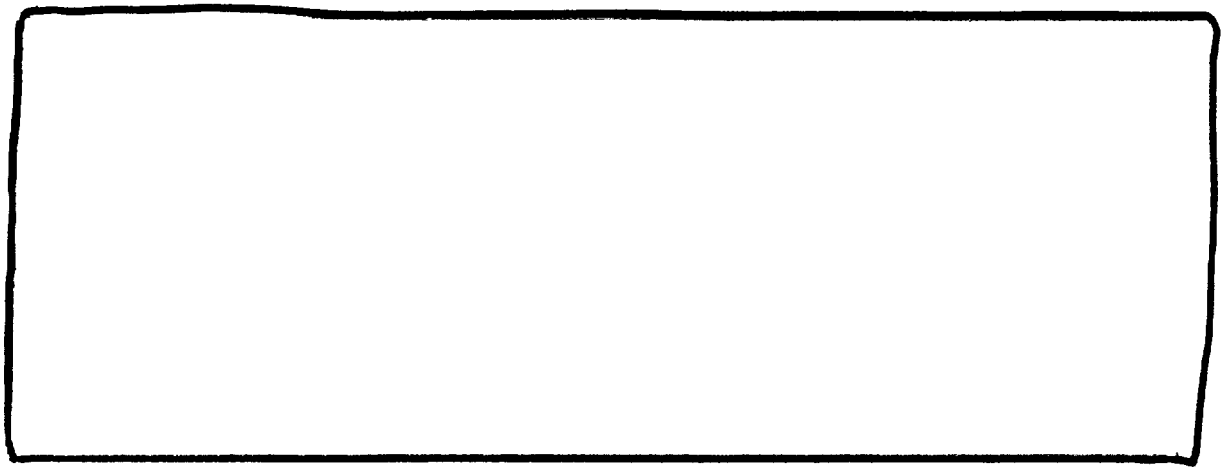
Incidence of several ovary changes in female rats after being treated with AGN192024

Dosage (mg/kg/day)	0	4	8	16
Terminal kill				
Number of pairs of ovaries examined	10	10	10	10
Prominent corpora lutea	0/10	6/10	8/10	10/10
Cellular vacuolation in corpora lutea	0/10	10/10	10/10	10/10
Slight	0	5	1	0
Moderate	0	4	6	0
Marked	0	1	3	10
Mean number of vacuolated corpora lutea	0	67.79	78.77	90.85
Mean total number of corpora lutea	36.46	74.88	86.63	99.26
Recovery kill				
Number of pairs of ovaries examined	5	5	5	5
Prominent corpora lutea	0/5	0/5	0/5	2/5
Cellular vacuolation in corpora lutea	0/5	4/5	5/5	5/5
Mean number of vacuolated corpora lutea	0	6.82	10.95	27.33
Mean total number of corpora lutea	39.79	52.73	49.45	63.43

Organ weights: ovary weight in treated animals was increased (see table below). No other toxicologically significant differences were noted.

Ovary weight in rats treated with AGN192024 for 13 weeks (mg)

Dosage (mg/kg/day)	0	4	8	16
Terminal kill	86.1	129.8	158.8	166.0
Recovery kill	102.1	104.9	92.8	93.1



Conclusion: Rats were treated orally with AGN192024 (4, 8 or 16 mg/kg/day) for 13 weeks followed by a 4-week recovery period. The decreases in terminal body weights and body weight gain were noted. ALT and AST activities were increased (2- to 5-fold) in male rats. Prominent corpora lutea (total of more than 70 in both ovaries), vacuolated corpora lutea and increased ovary weights were noted in females at all doses. The sponsor claimed that the mechanisms of the ovarian changes were not known, but these changes were likely related to pharmacologic effects of luteolysis by $PGF_{2\alpha}$, and were considered species-specific. All treatment-related changes showed reversibility during the recovery period.

13. AGN 192024: Toxicity study by oral gavage administration to CD rats for 52 weeks followed by an 8-week recovery period. Vol. 21, Page 147

Study N[o]: ALG 056/992437
Study Site: [redacted]
Compound: AGN192024 (0.2%, Lot#s: 11455, 11482 and 11483, Purity = 96-100%)
Vehicle: [redacted]
Animal: Crl:CD(SD) IGS BR rats, 6 weeks old, 147-197 g for ♂; 126-177 g for ♀)
Route: Oral (gavage)
Study Initiation: March 2, 1999
GLP/QAU: Yes

Dosing regimen for Study ALG 056/992437

			Number of rats (n/sex/group)			
Group	Dosage (mg/kg/day x 52 weeks, po)	Dose volume	Main	Recovery	TK	Total
1	Vehicle control	2 ml	15	5	6	26
2	0.1	2 ml	15	5	6	26
3	0.3	2 ml	15	5	6	26
4	2.0	2 ml	15	5	6	26

The purpose of this study was to evaluate toxic effects of AGN 192024 administered by gavage for 52 weeks followed by an 8-week recovery period in rats. Toxicity was assessed as shown below.

Toxicity assessment for Study ALG 056/992437

Parameter	Procedure
Mortality and clinical observations	At least twice daily
Body weights	Weekly
Food consumption	Weekly
Ophthalmic examinations	Predose, Week 51 of treatment and Week 7 of recovery. Indirect ophthalmoscopy and slit-lamp biomicroscopy examinations were performed on main and recovery animals.
Clinical pathology	Blood and urine samples were collected from all main study and recovery rats in Weeks 13, 26 and 52 of treatment and week 8 of recovery.
Gross pathology	At the end of the treatment or recovery periods, all of the main study or recovery rats were euthanized and necropsied.
Histopathology	The following tissues from all animals in Groups 1 and 4, and the liver and ovary from all animals were examined histologically: adrenals, aorta, brain, cecum, colon, duodenum, epididymis, eyes, harderian gland, head, heart, ileum, jejunum, kidneys, lachrymal glands, liver, lungs, lymph nodes (mandibular and mesenteric), mammary area, esophagus, ovaries, pancreas, pituitary, prostate, rectum, salivary gland, sciatic nerve, seminal vesicles, skeletal muscle, spinal cord, spleen, sternum, stomach, testes, thymus, thyroids with parathyroids, tongue, trachea, urinary bladder, uterus with cervix, vagina and any gross lesions.
Organ weights	Organ weights were obtained for the following in each animal: adrenals, brain, epididymides, heart, kidneys, liver, lungs, ovaries, prostate, pituitary, salivary glands, seminal vesicle, spleen, testes, thymus, thyroid with parathyroids, uterus with cervix.

[redacted]

Results:

Clinical observations: No treatment-related mortality and clinical signs were observed.

Body weights: No treatment-related changes in body weights or body weight gain were noted in males. However, reduced body weight gain was seen in females at 0.3 and 2.0 mg/kg (see table below).

Body weight changes in females caused by AGN 192024 administered orally for 52 weeks (g)

Mean body Weights	Dosage (mg/kg/day)			
	Control	0.1 mg/kg	0.3 mg/kg	2.0 mg/kg
Week 52	407	423	386	369
% of control	100	104	95	91
Week 60	422	442	394	361
% of control	100	105	93	86
Body weight gain				
Weeks 0-52	263±55.6	277±76.1	240±32.2	225±45.9
% of control	100	105	91	86
Weeks 52-60	-6.7±19.6	14.5±21.4	3.5±9.3	10.5±14.3

Food consumption: In female animals, a slight decrease in food consumption (4-5%) was noted at 0.3 and 2.0 mg/kg/day. It might not be toxicologically significant since the decrease was so small. No increase in the efficiency of food utilization (food consumed/body weight gain) was seen in these animals (6.3) relative to the control animals (6.7).

Ophthalmoscopy: No treatment-related changes were observed.

Clinical chemistry: An increase in ALT and AST activities was noted in most treated groups (see table below). Since the increase was small, reversible and was not associated with histopathological changes, these changes might not be toxicologically significant. No other toxicologically significant differences were noted.

ALT and AST activities in rats treated with AGN 192024

	Males				Females			
mu/ml	Dosage (mg/kg/day)							
Week 13	Control	0.1	0.3	2.0	Control	0.1	0.3	2.0
ALT	46±15.3	41±6.9	49±28.8	60±27.3	33±9.6	31±5.8	32±7.3	33±9.0
AST	75±16.2	74±13.4	81±29.1	89±30.0	64±10.1	61±6.3	69±22.6	68±7.9
Week 26								
ALT	62±51.2	66±54.1	71±96.2	195±223.1	38±16.8	47±27.2	46±15.8	76±69.7
AST	79±28.5	83±45.5	87±71.1	172±156.9	61±17.5	76±32.1	86±36.6	111±81.2
Week 52								
ALT	99±139.1	249±317.3	226±225.6	184±135.1	44±19.0	54±23.2	42±11.4	78±57.7
AST	125±96.9	200±165.5	212±156.2	183±100.3	90±23.2	109±69.0	90±23.3	123±71.9
Week 60								
ALT	135±190.8	48±4.8	66±50.4	48±7.2	64±30.7	58±37.1	54±15.9	51±17.3
AST	147±135.7	74±8.3	158±173.5	89±12.2	107±26.9	95±37.1	126±67.0	82±21.7

Hematology: No toxicologically significant differences were noted.

Urinalysis: No toxicologically meaningful events were noted.

Gross pathology: No treatment-related findings were noted.

Histopathology: Cellular vacuolation in a proportion of corpora lutea was found in 1 rat at 0.3 mg/kg and 3 rats at 2.0 mg/kg (see table below). In recovery animals, vacuolated corpora lutea was only seen in 1 female at 2.0 mg/kg, and the proportion of corpora lutea with cellular vacuolation in this rat was less than that seen in the main study animals, indicating that these changes were reversible after 8 weeks recovery.

Incidence of ovary changes in female rats after being treated with AGN192024 for 52 weeks

Dosage (mg/kg/day)	0	0.1	0.3	2.0
Total number of ovaries examined	14	15	15	15
Number of ovaries with corpora lutea	4	6	9	3
Cellular vacuolation in corpora lutea	0	0	1	3

Organ weights: No toxicologically significant differences were noted.

[redacted]

In summary, rats (Crl:CD(SD) IGS BR) were treated orally (by gavage) with AGN192024 (0.1, 0.3 and 2.0 mg/kg/day) for 52 weeks followed by an 8-week recovery period. Cellular vacuolation in corpora lutea was seen in several rats at 0.3 and 2.0 mg/kg. Therefore, the ovaries were identified as the target organ for AGN 192024. The decreases in terminal body weights and body weight gain were also noted at the same doses in females. A small increase in ALT and AST activities was noted in the treated male and female rats with no corresponding histopathological findings. All treatment-related changes showed reversibility during the recovery period. The no observed adverse effect level (NOAEL) was considered to be 2.0 mg/kg/day for males and 0.1 mg/kg/day for females.

14. AGN 192024: A 1-week intravenous injection toxicity study in Sprague Dawley rats. Vol. 24, Page 001

Study N^{o}: TX97009
Study Site: Allergan, Inc., 2525 Dupont Drive, Irvine, CA 92713-9534
Study Purpose: To determine the maximum tolerated iv dose and the systemic exposure of AGN 192024 in rats
Compound: AGN 192024 0.2% solution [redacted]
Vehicle: [redacted]
Route: Intravenous injection
Animal: Sprague Dawley rats, 5-6 weeks old, 130-146 g for ♂ and 129-142 g for ♀, 3/sex/group
Study Initiation: April 29, 1997
GLP/QAU: No
Study Design:

Group	N/sex	Drug concentration	Dosage (mg/kg)	Dosing volume (ml/kg)
1	3	Placebo for 0.2%-	0	5
2	3	0.2%	5	2.5