

CENTER FOR DRUG EVALUATION AND RESEARCH

APPLICATION NUMBER: NDA 20-600

PHARMACOLOGY REVIEW(S)

6. APR 28 1996

Review and Evaluation of Pharmacology and Toxicology Data
Division of Dermatologic and Dental Drug Products, HFD-540

NDA: 20-600 (000); Related IND: IND

Dates Submitted, CDER Received, Assigned, Completed: 6/16/95; 6/21/95; 3/27/96

Sponsor: Allergan
2525 Dupont Drive
PO Box 19534
Irvine, CA 92713-9534
1-800-347-4500

Name of Drug: AGN 190168 (Tazarotene) 0.05%, 0.1% gel

Chemical Name: Ethyl 6-[(4,4-dimethylthiochroman-6-yl)ethynyl]nicotinate

Pharmacological Category: Retinoid

Indication: Plaque psoriasis and acne vulgaris

Route of Administration: Topical dermal

Recommended Dosage: Once/day

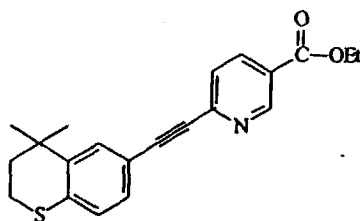
Formulation:

Ingredient: mg/g

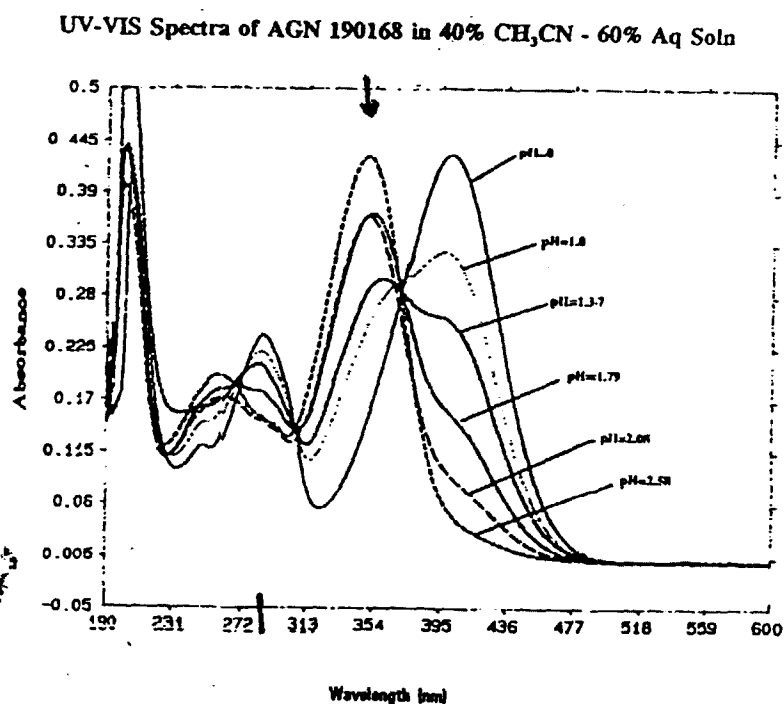
- Tazarotene
- Benzyl alcohol
- Ascorbic acid
- Butylated hydroxyanisole
- Butylated hydroxytoluene
- Edetate disodium
- PEG 400
- Hexylene glycol
- Carbomer 934P
- Tromethamine
- Poloxamer 407
- Polysorbate 40
- Purified water

(used in mfg. to deter oxidative decomposition)

Structure: MW 351.5



UV Spectrum: Tazarotene UV absorption shifts with increasing pH at and around Tazarotene's pKa value (1.5). The spectra below reveals three peaks at 269, 307, and 373 nm. The pH of the final clinical formulation is a range of 6.1-6.8. Dr. Wilson DeCamp, Supervisory Chemist in HFD-540, stated that the clinical formulation spectrum is likely to be quite similar to the spectrum at pH 2.6.



Index of Preclinical Toxicology Studies

ADME/Pharmacokinetics

Acute and Subacute

IV:

Single-dose IV injection study in rats; Study no. 1643-1894-4
Single-dose IV study in rabbits; Study no. 1643B-2970-5
Single-dose IV study in rabbits; Study no. 1643B-2970-10
Single-dose IV infusion study in dogs; Study no. 1643B-2970-11
Single-dose IV study in Cynomolgus monkey; Study no. 1643-WSRC-892211

Oral:

Minimum lethal oral dose study in rats; Study no. 1643-1177-11
4-week oral range-finding toxicity study in cynomolgus monkeys; Study no. 1643-WIL-123007

Dermal:

Single-dose skin toxicity study in rats; Study no. 1643B-2667-17
Single-dose dermal study in rabbits; Study no. 1643B-2679-13
Single-dose dermal study in rabbits; Study no. 1643B-2679-14
Single-dose dermal study in rabbits; Study no. 1643B-2667-15
Single-dose dermal study in rabbits; Study no. 1643B-2667-16
One-month abraded skin toxicity study in rats; Study no. 1643-1724-3
4-week dermal study in mice; Study no. 1643-ALG/27-931926
One-month skin toxicity study in rats; Study no. 1643B-2679-1
10-day comedogenicity study in rabbits; Study no. 1643B-2970-8
Dermal sensitization study in guinea pigs; Study no. 1643-IRDC-235-014

Ocular:

Acute single-dose ocular study in rabbits; Study no. 1643B-2970-3

Photo:

Phototoxicity in guinea pigs; Study no. 1643-IRDC-235-013
Photoallergy in guinea pigs; Study no. 1643-PH458-AN-001-94

Subchronic Studies:

Oral:

90-day oral toxicity study in rats; Study no. 1643-BTC-PO1768
13-week oral toxicity study in cynomolgus monkeys; Study no. 1643-WIL-123008

Dermal:

13-week dermal toxicity study in mice; Study no. 1643-ALG/22-931378
3-Month dermal toxicity study in minipigs; Study no. 1643-TPS-440A-601-234-91

Chronic Studies:

Oral:

26-week dietary study in rats; Study no. 1643-ALG/21-931051
6-month oral toxicity study in monkeys; Study no. 1643-HWA985-120

Dermal:

6-month dermal toxicity study in rats; Study no. 1643-1725-2

12-month dermal toxicity study in miniswine; Study no. 1643-TPS440B-602-244-91

Reproduction

Rats:

Dermal range-finding teratology study in rats; Study no. 1643-SLS-3202.4A-

- Dermal teratology study in rats; Study no. 1643-SLS-3202.1

Dermal range-finding teratology study in rats with Retin-A; Study no. 1643-SLS-3202.4B

Dermal teratology study in rats with AGN 190168 and Retin-A; Study no. 1643-SLS-3202.5

Dermal fertility and reproduction study in rats; Study no. 1643-SLS-3202.7

Dermal peri- and postnatal study in rats; Study no. 1643-SLS-3202.10

Range-finding developmental toxicity study in rats; Study no. 1643-SLS-3202.11

Developmental toxicity study in rats; Study no. 1643-SSL-3202.12

Rabbits:

Dermal range-finding teratology study in rabbits; Study no. 1643-SLS-3202.8

Dermal teratology study in rabbits; Study no. 1643-SLS-3202.9

Range-finding developmental toxicity study in rabbits; Study no. 1643-SLS-3202.13

Developmental Toxicity Study in rabbits; Study no. 1543.SLS-3202.14

Mutagenicity

Ames/Salmonella Plate Incorporation Assay; Study no. 1643-PH 301-AN-004-89

In vitro chromosome aberration analysis in human lymphocytes; Study no. 1643-PH 324-AN-001-90

E. coli liquid pre-incubation assay; Study no. 1643-PH 301-AN-001-93

CHO/HPRT Mammalian cell forward gene mutation assay; Study no. 1643-PH 314-AN-002-94

In vivo micronucleus test in mouse bone marrow erythropoietic cells; Study no. 1643-PH 309-AN-003-93

Carcinogenicity

Range-finding test for Photocarcinogenicity; Study no. 1643-C-1801-001P

12-Month Photocarcinogenicity in Hairless Mice; Study no. 1643-C-1801-001P

13-Week Oral Dose-finding Study in Rats; Study no. 1643-ALG/18-920710

2-Year Dietary Study in Rats; Study no. 1643-ALG/19/943062

Introduction

Tazarotene was first submitted as an original IND in 1990. The active ingredient is a new retinoid, an ethyl ester. The active form of the drug is the free acid, which is also the primary metabolite (AGN 190299). Tazarotene is the first topical retinoid intended to treat plaque psoriasis submitted to the agency as an NDA; Tazarotene is also being developed for acne vulgaris. The exact mechanism of action for retinoids remains unknown, although evidence suggests a three-fold effect in acne: the drug decreases cohesiveness of follicular epithelial cells and thus decreases microcomedo formation; stimulates mitotic activity and increases turnover of follicular epithelial cells, which tends to cause extrusion of comedones; and third, the drug decreases sebum production.

In plaque psoriasis, retinoids appear to intercede in the hyperproliferation of keratinocytes, loss of differentiation, and inflammation. Tazarotene blocks epidermal ornithine decarboxylase (ODC) in mouse epidermis; ODC is increased in psoriatic plaques and is associated with cell proliferation and hyperplasia. Tazarotene may also down regulate skin inflammation (as demonstrated by markers ICAM-1 and HLA-DR), and suppresses neutrophil chemotactic factor (MRP8) in vitro; MRP8 is overexpressed in psoriatic skin. Tazarotene, like other retinoids, appears to inhibit tumor cells, such as human tumor xenografts in nude mice, in vitro human tumor samples, Kaposi's Sarcoma cells, and ectocervical cell lines transformed with human papilloma virus.

Tazarotene does not bind nuclear retinoic acid receptors. The free acid metabolite, AGN 190299, binds and regulates gene expression through the nuclear retinoic acid receptor family ($RAR\alpha\beta\gamma$), with selectivity for $RAR\beta$ and $RAR\gamma$. Tazarotene and AGN 190299 are inactive in the second family of known retinoid receptors, the RXR family, in in vitro binding studies. (RAR and RXR are DNA binding proteins, and are members of the thyroid/receptor superfamily.) Tazarotene appears to have no effect in other major receptor populations, including acetylcholine, histamine, nicotinic, or serotonin receptors.

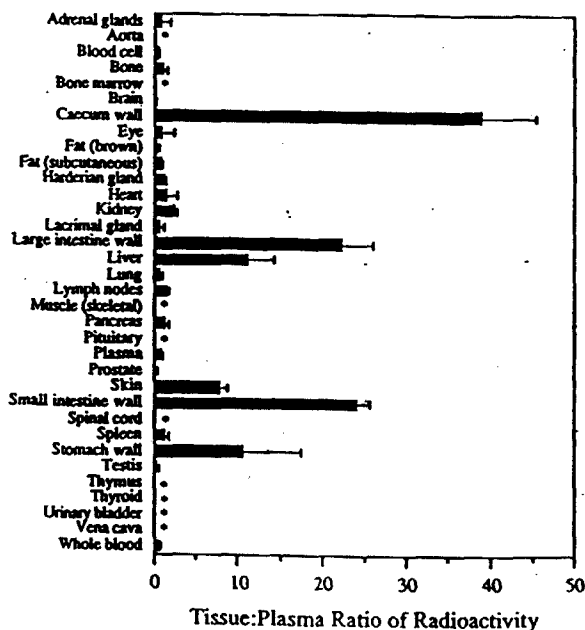
Expected adverse reactions associated with any topical retinoid include sometimes severe skin irritation, although the skin reactions are reversible. Tretinoin (Retin-A[®]) is considered teratogenic in animals (pregnancy category C). Carcinogenicity testing for Renova[®] (a soon-to-be-marketed topical tretinoin formulation) was negative, although the assay for photocarcinogenicity in CD-1 mice was positive. The expected clinical dosage of the 0.1% Tazarotene gel is 0.1 mg (assuming 20% of body surface).

ADME/Pharmacokinetics

The ADME/PK studies are non-GLP studies performed in the sponsor's research laboratories; many are presented in only a summary form. The known hydrolytic and oxidative pathways of Tazarotene (AGN 19068) are shown on the following page. AGN 190299 is the primary and only pharmacologically active metabolite formed; this free acid metabolite formed through hydrolysis was also produced in in vitro skin studies (fuzzy rat, human, and cadaver skin). The free acid is further metabolized to a sulfoxide, which is excreted in urine. Enzyme induction and inhibition studies were negative for common hepatic enzymes such as P-450.

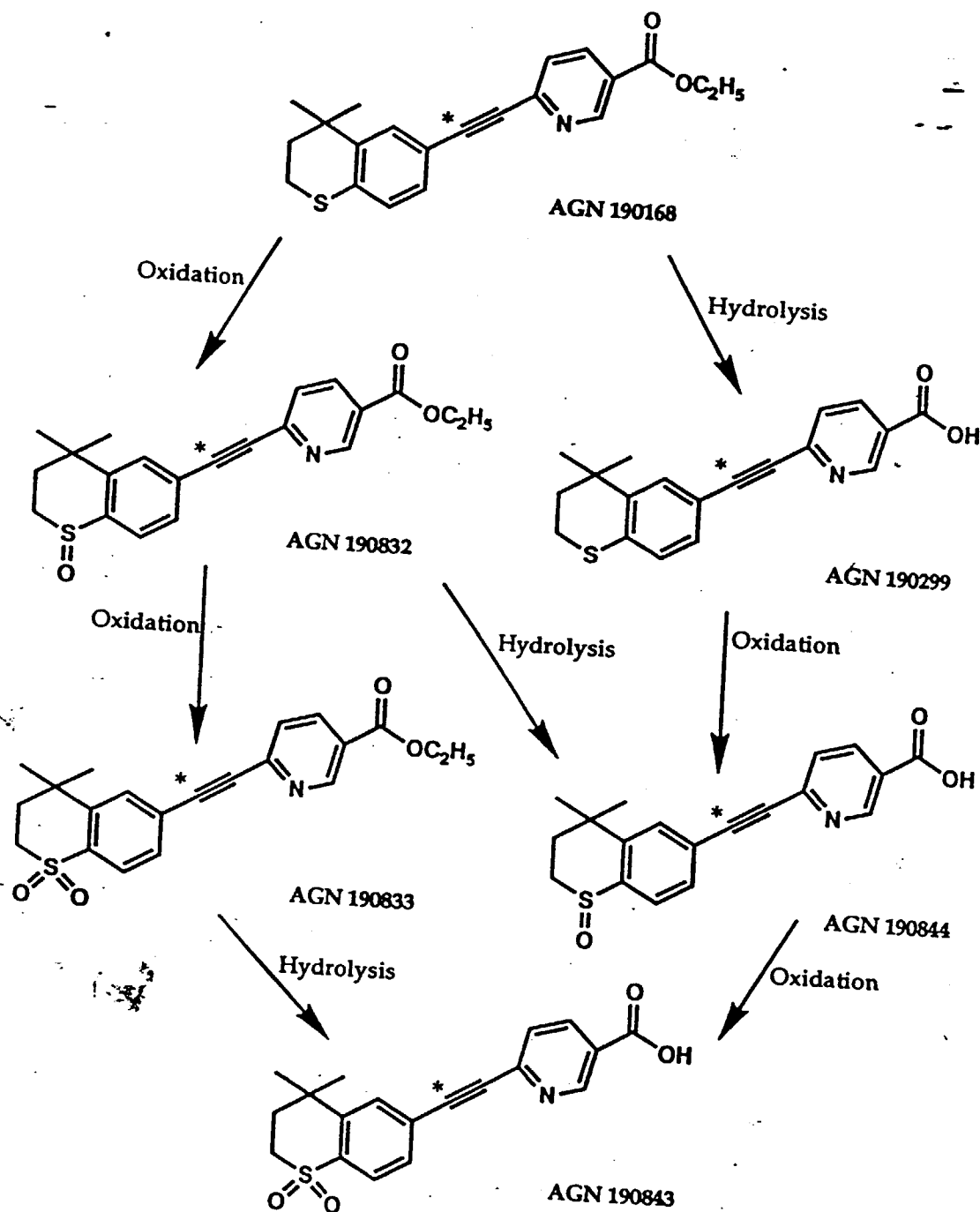
Radiolabel drug studies of blood to plasma concentration in mice, rats, rabbit, and humans indicated that the drug or metabolites were highly bound to plasma proteins. Mean unbound drug was less than 1% in in vitro human and in vitro and in vivo animal studies. Radiolabel drug skin disposition studies in male miniswine revealed percutaneous absorption of 7.4% in the viable epidermis (the putative site of action). In a topical study to examine tissue distribution in rats, 21-day topical administration of 0.1% radiolabeled gel resulted in a general distribution throughout the body. Highest levels were found in the skin, liver, and intestinal tract. Radioactivity was noted rapidly in the liver and gastrointestinal tract after topical dosing, which suggests that elimination may occur through biliary excretion. (As in all topical studies, there is no way to accurately estimate the amount of compound that may have been ingested during grooming and the amount that penetrated the skin and entered the systemic circulation.) Results are summarized in the figure below.

Tissue:plasma Ratio of Radioactivity in Tissues After 21 Days of Repeated Topical Administration of ^{14}C -AGN 190168 to the Skin of Male Rats



Values are mean \pm standard deviation, $n = 3$. * = no radioactivity detected in tissues. Tissue samples taken 24 hours after 21 days of topical dosing.

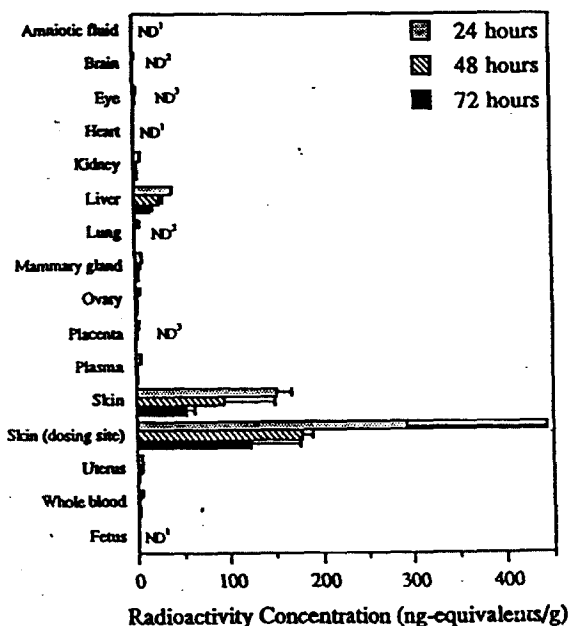
The Known Hydrolytic and Oxidative Pathways of AGN 190168 Metabolism
(The * denotes the position of the ^{14}C -label.)



Following IV dosing in rats, radioactivity was first noted in the liver, plasma, and small intestine (0.5 hours); and by 8 hours radiolabel was found in the spleen, large intestine, cecum, liver, and ovaries. By 48 hours, radioactivity was still noted in the spleen, liver, adrenals and ovaries. No sex differences in disposition were noted. Autoradiography of rats following topical dosing also revealed radioactivity in the skin, gastrointestinal tract, buccal mucosa, and liver (24 hours); 192 hours post dose showed that the drug was virtually cleared.

An autoradiography study in pregnant rats gave similar results and revealed no placental transport (see figure below). A placental transfer study following an oral dose on gestation day 18 in pregnant rats revealed radioactivity absorbed and distributed throughout maternal tissues. Fetal tissues had much lower ranges than maternal tissues: 7.1 - 14.2 ng-eq/g compared to 53.5 - 287.1 ng-eq/g. Similar studies were carried out in pregnant rabbits, and fetal tissue levels were also much lower than maternal tissue levels (1.2 ng-eq/g compared to 57.3-78.8 ng-eq/g). A single topical application of radiolabeled AGN 190168 (0.19 mg/kg) applied to nursing rats resulted in secretion in milk from 8 to 48 hours. These data indicate that a single topical application would result in drug being passed in the milk of lactating rats.

Concentrations of Radioactivity (ng-eq/g) in Tissues After Daily Topical Administration of ^{14}C -AGN 190168 on Days 6 - 15 of Pregnancy to the Skin of Female Rats



Values are mean \pm standard deviation, n = 3. ND¹ = no radioactivity detected at 24, 48 and 72 hours; ND² = no radioactivity detected at 48 and 72 hours; ND³ = no radioactivity detected at 72 hours.

The sponsor performed a wide range of PK/TK studies in numerous species with different dosing regimens, including topical, IV, IP, subcutaneous, oral, and dietary. Dermal topical studies are summarized in the tables on the following pages, and data submitted with individual toxicology studies are reviewed with the study. The sponsor also submitted PK studies using an IV dosing regimen. These IV studies are presented with similar topical studies for comparison. The key is presented below the last page of the table.

Topical Day 100 mg/kg												
Species n/sex	Age for Study	Dose mg/kg	Time (hours)	Conc (mg/ml)	ALT mg/hr/ml	ALT mg/hr	Vol ml/kg	ALT mg/hr	ALT % of dose	ALT % dose	ALT % dose	Reference
mouse 5/sex	4 weeks	0.50 1.00		47 (23) 72 (20)								PK-94-022
mouse 3/sex	13 weeks	0.050 0.10 0.25 0.50		13 (2) 20 (5) 65 (18) 141 (80)								PK-94-020
mouse 3/sex	every other day for 13 weeks	0.050 0.10 0.25 0.50		13 (3) 26 (5) 42 (11) 67 (6)								PK-94-020
mouse 2-5/sex	16 weeks	0.050 0.10 0.25 0.50	3 3 2.5 3.5	12 24 29 99	64 130 160 450							PK-95-037- ALG-25
mouse 2-5/sex	53 weeks	0.050 0.10 0.25 0.50 m 1.00 f		9 13 29 25 85								PK-95-037- ALG-25
mouse 38/sex	IV	1.65 m 1.49 f	0.03 0.03	3950 3580	43, 6900 55, 7450	0.64 0.45	3.43 0.98	0.06, 1.6 0.03, 1.6				PK-1992- 074

Species n/sex	Route of study	Dose mg/kg	Time (hours)	Concn (mg/ml)	Alt mg/ml	Time min	Vol L/kg	Alt/2 hr	Alt % of dose	Alt % dose	Alt % dose	
rat 45 males	1 x (normal skin)	0.15							4.4 (0.7)	35 (9)	40	PK-1989- 033
rat 42 males	1 x (abraded skin)	0.17							3.5 (0.3)	33 (2)	37	PK-1989- 033
rat 16 females	1x	0.18		10 (1) ^a				38 ^{a, b}	4.9 (2.1)	61 (25)	66	PK-94-024
rat 36 males	7 days	0.063		2.6 (1.3) ^a				2.72 ^a days	9.4 (0.8)	77 (27)	86	PK-1993- 024
rat 5/sex	28 days	0.125 0.250 0.125 0.250	2 (1) 3 (1) 2 (1) 3 (1)									PK-94-009
rat 5- 6/sex	1 day	0.1250 0.250	4.7 11	12 (5) 9.7 (2)	65 (15) 74 (11)							PK-94-090
rat 5- 6/sex	28 days	0.125 0.250 0.125 0.250	11 10 10 9.5	3.4 (0.5) 3.9 (0.4) 4.5 (0.8) 6.4 (1.2)	46 (4) 54 (5) 46 (2) 71 (5)							PK-94-090
rat 2 f	iv	1.2 5.0	2 min	950 8600	700 8700	0.03 0.01	0.5 0.5					PK-1989- 007

Species n/sex	Off Study	Dose mg/kg	Times (hours)	Chgs (mg/ml)	Alt (mg/ml)	Wt g/kg	Alt g/kg	Alt g/kg	Alt g/kg	Alt g/kg	Alt g/kg	Alt g/kg
rat 5- 7/sex	28 days 2 x/day	-0.03 -0.2 -0.4	15 m 14 f 8.1 m 10 f 11 m 7.8 f	0.73 (0.28) 1.2 (1.1) 5.5 (3.0) 4.2 (2.5) 6.1 (2.5) 4.9 (0.7)	8 (3) 13 (9) 52 (19) 50 (15) 72 (20) 80 (16)							PK-95-033
rat 8 PG females	gestation days 6- 17	0.25	13	9.8 (5.8)	107 (49)							PK-95-034
rabbit 5 PG females	gestation days 6- 18	0.125 0.250 0.125 0.250	0.1 (0.02) 0.3 (0.4) 0.2 (0.1) 0.6 (0.3) parent - BLQ									PK-1993- 043,SLS320 2.8
rabbit 7 PG females	gestation days 6- 18	0.25	9.7	86 (20)	1160 (290)			48 (9)				PK-95-034
rabbit 3 M	iv	1.9	0.17	11000	138, 37800	0.25 0.000 8	2.1 0.2	0.1 2.2				PK-1992- 074

Species in sex	Age of Study	Dose mg/kg	Time (days)	Conc. (µg/ml)	Alt (mg/100ml)	Alt (mg/kg)	Vd (l/kg)	Cl/2 (hr)	Alt % of dose	Alt % dose	Alt % dose	Study
mini- swine 2/sex	91 days	0.05 0.125 0.25 0.50	8.0 (3.3) 5.0 (2.0) 5.0 (2.0) 4.0 (0)	0.4(0.08) 0.9 (0.3) 1.3 (0.1) 2.2 (0.5)	6.4 (1.5) 14.9 (5.1) 22.1 (1.8) 37.6 (13.5)							PK-1993- 008, TPS440A
mini- swine 6 males	13 weeks 2 x/day	0.05 0.125 0.25		2.3 (1.1) 2.0 (0.5) 1.5 (0.8)								PK-94-017, TPS440B
"	26 weeks 2 x/day	0.05 0.125 0.25		1.3 (0.5) 2.5 (2.1) 2.4 (1.0)								"
"	52 weeks 2 x/day	0.05 0.125 0.25		0.6 (0.2) 1.1 (0.3) 1.2 (0.5)								"
mini- swine 6 females	13 weeks 2 x/day	0.05 0.125 0.25		0.8 (0.3) 1.9 (0.7) 2.2 (1.1)								"
"	52 weeks 2 x/day	0.05 0.125 0.25		0.5 (0.4) 0.9 (0.5) 0.9 (0.4)								"
"	26 weeks 2 x/day	0.05 0.125 0.25		0.8 (0.3) 1.9 (0.7) 1.9 (0.7)								"
miniswine 4 M	iv	0.5	0.1	713	366, 1010	0.03 0.01	8.2	5.73 11.0				PK-94-055

Species n/sex	Dose mg/kg	Dose mg/kg	Concn (ng/ml)	Concn (ng/ml)	AUC (ng·h/ml)	Cl _{CR} (ml/min)	V _d (L/kg)	t _{1/2} (h)	Aur % of dose	Afe % of dose	F % dose	
Monkey 4 males	1 x	0.17		blq					1.7 (0.6)	1.8 (1.0)	5.3 (1.6)	PK-1989- 036, PK- 1990-022
monkey 4 males	1 x	0.17	blq						1.2 (0.5)	0.89 (0.3)	2.1 (0.4)	PK-1991- 031
monkey, 4 M	iv	0.5	0.2	1240	186 1710	0.1	1.3	0.3 1.5				PK-1989- 035

Numbers in italics are for quantification of the metabolite AGN 190299

based on total reactivity

half-life estimate is based on biliary excretion and terminal plasma levels

Aur = total urinary excretion

Afe = total fecal excretion

F = systemic bioavailability

n = male

♀ = female

blq = below level of quantification

All studies were dosed topically, unless otherwise indicated.

Toxicology Studies

Acute, Subacute, and Special Studies

IV:

Single-dose IV injection study in rats; Study no. 1643-1894-4; Allergan; 12/89

Sprague Dawley rats (24/sex) were injected IV with 2 mg/kg 0.02% AGN 190168. Animals were observed for 7 days; no dose-related differences in clinical observations or body weights were noted between the treated and control groups.

Single-dose infusion IV studies in rabbits; Study no. 1643B-2970-5 and 1643B-2970-10; Allergan; 4/94 and 12/94, respectively

New Zealand rabbits were given by IV infusion 0.015 and 0.075 mg/kg 0.05% Tazarotene in % ethanol and observed for 14 days. No compound-related differences were noted in body weights, clinical signs, or blood chemistry values. In the second study, rabbits were dosed with 0.012 and 0.060 mg/kg 0.01% AGN 190168, although due to adhesion to the infusion tube the dose levels were 20-25% less than planned. No dose-related effects were noted in clinical observations, body weights, blood chemistry, or necropsy observations following 14 days of observations after dosing.

Single-dose IV infusion study in dogs; Study no. 1643B-2970-11; Allergan; 12/94

Beagle dogs (6/sex) were dosed by IV infusion with 0.012 and 0.060 mg/kg in males and 0.015 and 0.075 mg/kg in females 0.01% AGN 190168, and observed for 14 days prior to the end of the study. (Female doses were increased following the realization that 15-20% of compound was adhering to the tube.) No compound-related effects were noted in clinical observations, body weights, blood pressure and pulse rate, hematology, and blood chemistry. Animals were not necropsied.

Single-dose IV study in cynomolgus monkey; Study no. 1643-WSRC-892211; (Study 89221); 12/89

Cynomolgus monkeys (1/sex) were given by IV bolus 0.75 mg/kg AGN 190168 (3 ml/kg of a 0.025% solution); two additional monkeys (1/sex) were given a placebo IV. Animals were observed for 1 week following treatment. No compound related changes in clinical observations, body weights, or behavior were noted.

Oral:

Minimum lethal oral dose study in rats; Study no. 1643-1177-11; Allergan, 9/89

Sprague-Dawley rats (10/sex) were given a single oral dose of 20 ml/kg 10% AGN 190168, and were observed for 14 days. Clinical observations included lethargy, piloerection, soiled perianal region, slight paraphimosis, blood around the nose, hair loss, and bloody tears. All animals survived until day 14, and no compound-related findings were noted in body weights or at necropsy.

4-week oral range-finding toxicity study in Cynomolgus monkeys; Study no. 1643 123007; 12/89

Cynomolgus monkeys (1/sex) were dosed with 1.0, 2.5, 5, or 20 mg/kg AGN by oral intubation for 4 weeks. Signs of mortality, morbidity, and severe toxicity were noted at all dose levels but the lowest dose (1.0 mg/kg). On days 8-9, animals were found dead or sacrificed in extremis in the high-dose group (20 mg/kg), and on days 17-18 in the 5 mg/kg group. One male died on day 13 in the 2.5 mg/kg group. Renal damage was indicated in creatinine, BUN, phosphorous, and calcium values by week 2 in the 5 and 20 mg/kg groups. At the microscopic examination, primary lesions were noted in the kidney, which included tubular nephrosis, suppurative inflammation, and tubular mineralization. Lesions said to be secondary to the renal damage included changes in the lung, spleen, heart, and stomach.

Dermal:

Single-dose skin toxicity study in rats; Study no. 1643B-2667-17; Allergan; 2/93
Sprague Dawley rats (5/sex/group) were treated with a single dose of 0.01 to 0.1 % AGN 190168 (0.1 ml) on the back, and observed clinically and for irritation for 14 days. No treatment-related differences were noted in body weights, clinical observations, or signs of irritation (erythema and edema). Unusual findings at necropsy, which include mottled kidneys and a swollen liver lobe, were noted in the treated and placebo groups.

Single-dose dermal studies (4) in rabbits; Study no's. 1643B-2679-13, 1643B-2679-14, 1643B-2679-15, and 1643B-2667-16, Allergan; performed 12/93, 7/94, 2/93, 6/93, respectively.

In the first two studies listed above, New Zealand white rabbits (6/sex) were given a single topical dose of 0.05 or 0.1 % AGN 190168 (0.1 ml) on the intact clipped backs of the rabbits; the area was occluded for 24 hours, and the animals observed for 14 days. Erythema, flaking/scaling, and edema were noted on drug-treated animals. The edema cleared by day 4; other observations decreased in number and severity over the 15-day observation period. Scabbing was noted on high-dose females. No lesions were noted at gross necropsy. In the last two studies listed above, rabbits (5/sex) were given a single topical dose of 0.01, 0.025, or 0.05 % AGN 190168 (0.1 ml) on shaved backs. The two studies differed in that the skin was slightly abraded with sandpaper prior to compound application. No unusual clinical observations were noted. On days 2-15, all AGN-treated males experienced slight to mild erythema. Males treated with 0.01 and 0.05 % AGN had one incidence each of edema; AGN 0.05 %-treated males had 2 incidences of flaking and scaling. All AGN-treated females experienced slight to mild erythema, and all female groups except the lowest dose experienced flaking and scaling; no edema was noted in the females.

In the other study, AGN was again a slight-to-mild irritant on rabbit skin despite the addition of abrasion.

One-month abraded skin toxicity study in rats; Study no. 1643-1724-3; Allergan; 12/89
Sprague Dawley rats (15/sex/group) were treated with vehicle, 0.1, 0.05, or 0.1 % AGN

190168 two times per day for 30 days on shaved, abraded animal backs. Three recovery groups were observed for an additional 30 days after treatment. By the fourth week of treatment, all drug-treated animals were noted with erythema (very slight erythema with a few incidences of severe erythema), fissures, scabs, flaking/scaling, and abrasion (very slight to mild). Vehicle-treated animals appeared normal throughout this period, except for a single incidence of abrasion (which was likely due to shaving prior to treatment). There was no clear difference in the incidence or severity of irritation among the three drug-treated groups. By week 8, the recovery groups appeared normal except for findings in the mid-dose animals: slight-to-mild erythema, slight fissures, scabs, slight flaking/scaling, and slight abrasion. No treatment-related changes in body weights, ophthalmologic exams, hematology, or gross necropsy were noted. (Hematology was evaluated at 31 days, and at 60 days in the recovery groups.) The skin was evaluated microscopically following necropsy. The non-treated and vehicle-treated animal skin appeared normal. Treated animals were noted with moderated epidermal pustule, hyperkeratosis, erosion, ulceration, acanthosis, severe subacute dermatitis, suppurative folliculitis, and superficial dermal fibrosis. Lesions increased in incidence and severity with dose level, although the mid- and high-dose groups were noted with very similar skin findings. In the recovery animals, no dose or treatment related effects were noted except for a single animal in each dose group.

4-week dermal study in mice; Study no. 1643-ALG/27-931926;

9/94

Mice (15/sex) were dosed dermally (3 cm² area) every day with vehicle, 0.50, or 1.0 mg/kg of 0.1% AGN 190168. No treatment related effects were noted in body weight, food conversion, and hematology values. Treated male mice were noted with significantly decreased total protein (albumin and globulin) and cholesterol values. At histopathology, high-dose mice were noted with changes in the liver (males, centrilobular hepatocyte enlargement) and stomach (females, epithelial hyperplasia and hyperkeratosis). High dose males and females were noted with changes in the skin that included erythema, minimal erosion, epithelial acanthosis and hypertrophy. The sponsor also performed pharmacokinetics in this study. Blood samples were taken 2 hours after the final dose. Levels of AGN 190299 (the primary metabolite of the parent compound) were 0, 47.1 ± 22.5 , and 71.5 ± 19.9 ng/ml for groups 1-3, respectively. Note that there appeared to be extreme variability of blood concentration, which varied from ng/ml for treated animals. Sensitivity of the assay was ng/ml.

One-month skin toxicity study with a recovery period in rats; Study no. 1643B-2679-1, Allergan, 3/95

This study served as a bridging study between formulation changes. Sprague-Dawley rats were dosed twice daily (3 cm² area) with 0.05 (0.125 mg/kg/day) or 0.10% (0.25 mg/kg/day) AGN 190168 and then allowed a two-week recovery period. The 0.05% preparations 8225X and 8607X were compared; and the 0.10% preparations 7997X and 8606X were compared. The "old" formulations, 8225X and 7997X contain approximately 0.5% less water and triethanolamine (trolamine), rather than tromethamine. The "new" formulations, 8606X and 8607X, contain tromethamine and are made up of the same ingredients as the final clinical

formulation. Skin findings consisted primarily of minor skin irritation, and microscopic changes (hyperkeratosis, epidermatitis, dermatitis, perifolliculitis) diminished in severity after the recovery period. There appeared to be no difference in severity of irritation between the two formulations, although increased dose of the active ingredient resulted in increased irritation in both formulations and females appeared to be somewhat more effected. Treated females also had decreased body weight gains. A variety of slight to moderate changes in clinical chemistry and hematology values were noted; no differences were noted between the two formulations and virtually all values returned to normal following the 2-week recovery period. No treatment-related systemic changes were noted in the histopathological exam. Animals were examined for pharmacokinetic parameters following 4 weeks of treatment (2 hours post dosing). Blood levels of AGN 190299 (the major metabolite) ranged from 1-4 ng/ml. No differences in blood levels were noted between formulations. Females blood levels were lower than male values, sometimes as much as half. Low concentrations of AGN 190299 were detected in the blood of several vehicle control animals; the sponsor did not explain this cross contamination.

10-day comedogenicity study in rabbits; Study no. 1643B-2970-8, Allergan, 9/94
AGN 190168 (0.05 or 0.1%) was applied to the inner surface of the ear pinna (0.05 ml) for 10 days to test for comedogenicity. Both concentrations caused slight enlargement of the hair follicles when examined by whole mount technique. The follicles showed small globoid masses of horny material. In the positive control group treated with isopropyl myristate (IPM), slight to moderate follicle enlargement was noted. The treated animals also were noted with well-defined erythema, slight flaking/scaling, scabs, and abrasions. In the histological examination, AGN-treated animals were noted with the inflammatory cell periglandular infiltration, atrophy, and displacement of the sebaceous glands; inflammatory cell infiltration, hyperkeratosis and edema of the external root sheath of the hair follicle; and epidermal hyperkeratosis, edema, hemorrhage, and inflammatory cell infiltration in the dermis and epidermis. The sponsor stated that they believed the changes were indicative of skin irritation, and that AGN 190168 did not cause a classical comedo response.

**Dermal sensitization study in Guinea pigs; Study no. 1643 235-014;
12/89**

AGN 190168 (0.01, 0.05, or 0.1%) was administered to guinea pigs (10/group) under occlusion weekly for 3 weeks. The animals were challenged 2 weeks after the final induction exposure using the same test material. No erythema was noted within 48 hours after challenge on any treated animal, and the treatment was not considered a sensitizing agent.

Ocular:

Acute single-dose ocular study in rabbits; Study no. 1643B-2970-3, Allergan, 3/94
AGN 190168 (0.05% and 0.1%, 0.1 ml) was applied to the left eye of New Zealand albino rabbits, and animals were observed for 14 days. The right eye served as the control. Moderate to severe ocular discomfort, tearing, and hyperemia was noted in all groups, including the

vehicle control, immediately after dosing. After approximately one-hour post dosing, no ocular reactions were noted for the remainder of the study.

Photo:

Phototoxicity in guinea pigs; Study no. 1643- 235-013; 12/89

Test material at dosage levels of 0.1, 0.05, and 0.01 % (0.15 ml) was applied to the shaved and depilated backs of Hartley guinea pigs. A positive control group was treated with 8-methoxy-psoralen. Test sites were occluded and left wrapped for 2 hours. One site was then covered with a light-protective patch and the other site remained uncovered and exposed to UVA (320-400 nm, 10 Joules/cm²) for approximately 1 hour. Positive control animals were noted with moderate erythema to delayed edema. No erythema or edema was noted in the AGN-treated groups after 0-96 hours of exposure.

**Photoallergy in guinea pigs; Study no. 1643- 458-AN-001-94;
3/95**

A dose-range-finding study was first performed; 10 guinea pigs were exposed to AGN 190168 (0.05 or 0.1%) for 30 minutes and were then irradiated with 10 J/cm² UVA. The other side of the animal was covered with an opaque UVA shield to test for contact allergy. Based on the minimal findings in this study, the 0.1% dose was chosen for the induction phase. In the induction phase, animals were treated with test article, placebo, or positive control (1% tetrachlorsalicylanilide; TCSA) 5 times over 15 days. After dose administration, guinea pigs were exposed to 30 J/cm² UVA radiation (102-140 seconds exposures, 310-400 nm). Animals were challenged 15 days after the last dosage; the elicitation phase applications duplicated the original exposure site and dose. One site was irradiated to test for a photoallergic response, and another site was not irradiated and it was evaluated for a simple contact allergic response. Skin treated with AGN gels appeared irritated (as measured by erythema), but did not have a contact sensitization reaction on irradiated or non-irradiated skin. The positive control group treated with TCSA was noted with moderately positive reactions at the irradiated and non irradiated sites.

Subchronic (less than 6 month) Studies

Oral:

**90-day oral toxicity study in rats with a 30-day recovery; Study no. 1643- PO1768;
12/89 and reissued with amendment 3/95**

Sprague Dawley rats (15/sex, 5/sex in recovery groups) were dosed daily by gavage with 0.05, 0.25, or 2.00 mg/kg for 90 days. Due to high mortality, a lower dose group (5/sex) was added 8 weeks after study initiation. In the 2.0 mg/kg day high-dose group, 14 females (11 from the main group and 5 from the recovery group) died or were sacrificed moribund. The animals were noted with concentrated urine, blood around their ear, nose, and mouth, weakness, and difficult breathing. Significant body weight decreases were noted in all animals

treated with drug, including the recovery animals. Alkaline phosphatase and ALT values were significantly higher and albumin, calcium and total protein were significantly lower in the high-dose (2.0 mg/kg) males and females. In high-dose females, AST, bilirubin, BUN, creatinine and phosphorous were increased; glucose was decreased. In males, globulin, potassium, and sodium values were decreased, and glucose was increased. Recovery animal clinical pathology values were comparable to control, except for decreased globulin and total protein in males.

Histopathology changes included bone (narrowing of the zone of proliferating cartilage, widening of the zone of maturing cartilage, multifocal chondrolysis), lung, thymus (involution, hemorrhage), heart (edema, epicarditis, chronic inflammation, and hemorrhage), and liver (hepatocellular hypertrophy, vacuolar change, extramedullary hematopoiesis). The changes were generally dose-dependent, and were not present in the lowest dose groups (0.05 mg/kg).

**13-week oral toxicity study in Cynomolgus monkeys; Study no. 1643 123008;
12/89**

Monkeys (4/sex/group) were dosed by nasal gastric intubation daily for 13 weeks with 0.05, 0.25, or 1.00 mg/kg AGN 190168. A recovery group (2/sex/group) remained on study for an additional 4-weeks following the dosing regimen. At week 6, the high-dose group dose was increased to 1.60 mg/kg day to achieve toxicity levels. In the high-dose group, one male died and 3 animals were sacrificed in extremis (1 male/ 2 females). Clinical observations for the high-dose group included prostration, hypothermia, inappetence, hypoactivity, decreased muscle tone and ocular discharge. The mid- and high- dose groups were noted with reduced body weights. In high-dose males the difference was 40% at week 13 and continued through recovery; high-dose female body-weight differences were approximately 20%. Platelet and APTT values were significantly higher in high-dose males, and did not recover 4-weeks post dose. In clinical chemistry values, increased BUN and glucose values and decreased albumin values and A/G ratios were noted in the high-dose males and females. In high-dose females, elevated creatinine and phosphorous were noted. In the histopathological exam, high-dose males that died prior to the scheduled sacrifice were noted with mineralization in the heart and/or kidneys, and two of the males, splenic lymphoid atrophy was noted. Thymic atrophy was noted in 4/8 high-dose males. One high-dose male exhibited myocardial serous atrophy and pneumonia. Although the pathologist did not consider it related to drug treatment, one moribund high-dose females was noted with a benign vascular tumor (hemangioma) in the liver. No abnormalities were noted in recovery females. Two high-dose recovery males and one control male were noted with aspermagenesis.

Dermal:

13-week dermal toxicity study in mice; Study no. 1643- /22-931378;
9/94

AGN 190168 (0.005, 0.01, 0.025, and 0.05%) was applied daily or on alternate days to the clipped backs of CD-1 mice for 13 weeks. No treatment-related deaths were noted. Erythema, dryness, and edema were noted in the 0.025 and 0.05% dose groups; the 0.25% dose group had fewer signs of irritation when dosed on alternate days. Edema was noted in 1-2 males/group and no females. Bodyweight gains over 13 weeks were statistically less than untreated control values for virtually all males treated every day, including control vehicle groups (30-50%). Males treated on alternate days had significantly decreased body weights in the 0.25 and 0.50% groups. Female body weights gains were comparable among the groups. No treatment related effects were noted for food consumption, food utilization, and water consumption. High-dose males had significantly increased eosinophils (10%); all other values were comparable for males and females. In high-dose males and the two highest doses in females, total protein was reduced (12% <). Other changes possibly indicative of liver effects included lower cholesterol values and triglycerides in males, and to a lesser extent in females. PK values (3 mice/sex/group) at week 13 revealed blood levels of 13.3 ± 2.4 ng/ml for the low dose animals to 141 ± 75 ng/ml for the high dose animal with daily dosing. In the alternate day dosing groups, the range was _____ ng/ml. No treatment-related organ weight changes were noted. Microscopic evaluation of the skin revealed epidermal erosion, acanthosis with hyperkeratosis, and epithelial hypertrophy. Incidence and severity increased with dose.

3-Month Dermal Toxicity and Irritancy Study in Minipigs; Study no. 1643- 440A-601-
234-91; 11/92

Animal Strain: Hanford Minipig

No. of Animals: 2 animals/sex/group

Route: Topical dermal

Duration: Once/day for 3 months

Study Design & Dose Levels:

Group	Test Material	Gel Applied g/kg	Active Drug mg/kg/day
1- Control	placebo	0.50	<u>0</u>
2-Low	0.025% AGN	0.20	0.050
3-Mid	0.05% AGN	0.25	0.125
4-Mid	0.1%	0.25	0.250
5-High	0.1%	0.50	0.500

Methods: Animals were exposed on the dorsal area of the trunk; exposure sites were kept clipped of hair. Test materials were applied daily to unoccluded skin and any remaining test material was removed prior to the next dosing.

Results:

Mortality: All animals survived until scheduled euthanasia.

Clinical Observations/Physical Observations: Numerous incidences of scabbiness and blackened skin were noted in the treated groups. Other findings were within the range of normal.

Body Weights: Body weights and body weight changes were comparable among all groups over the course of the study.

Dermal Irritation Results: Very slight to mild erythema was noted for all miniswine in Groups 3, 4, and 5 by the fourth week of the study, and by the fifth week of the study in Group 2. Unfortunately, after week 4 none of the Group 3, 4, and 5 animals could be evaluated because, according to the sponsor, "...a hard colored black scab had formed over the sites." Thus, dermal irritation cannot be assessed with this study.

Hematology (13 parameters): No significant differences were noted among groups for samples drawn in week 5. At the end of study, Group 5 male neutrophil count and Group 4 female eosinophil count were significantly lower than control values.

Chemistry (19 parameters): In week 5, group 5 male total bilirubin was significantly increased and group 4 blood urea nitrogen levels were significantly decreased when compared to control values. At the end of the study, blood urea nitrogen in group 5, albumin in groups 3 and 5, and albumin-to-globulin ratio (A/G) in group 3 in males were significantly less than control values. In females, group 4 A/G, and group 5 globulin and A/G were significantly less than

control values.

Ophthalmic Observations: No treatment-related findings were noted.

Organ Weights: No treatment-related differences in organ weights were noted.

Gross Necropsy Observations: Scabbing increased in severity with dose level and was noted on all treated animals; other observations were within the range of normal.

Histopathology: The skin from the treated areas of all treated groups had acanthosis with thickening stratum germinativum, inflammatory neutrophilic and cellular infiltration, cellular surface debris, and serum exudate and focal erosions in the epidermis. Groups 3, 4, and 5 animals also had focal ulcerations, focal fibrosis, and focal erosions in the epidermis (Groups 4 and 5 only). Observations increased in severity with dose level. Findings in all other organ systems were considered spontaneous and not treatment related.

Pharmacokinetic Evaluation: The following table summarizes pharmacokinetic parameters for the day 91 blood samples (n=4). Blood samples were measured at 0, 4, 8, 12, 16, 20, 24 hours post dose; no quantifiable concentrations of AGN or any metabolite were found in the placebo control animals.

Dose ($\mu\text{g/kg/day}$)	AGN 190299		
	C_{max} (ng/ml)	T_{max} (hour post dose)	AUC (ng•h/ml)
50	0.396 \pm 0.077	8.0 \pm 3.3	6.39 \pm 1.50
125	0.916* \pm 0.279	5.0 \pm 2.0	14.9* \pm 4.1
250	1.33* \pm 0.12	5.0 \pm 2.0	22.1* \pm 1.8
500	2.18* \pm 0.51	4.0* \pm 0.0	37.6 \pm 13.5

* Statistically different from the next lower dose ($p < 0.05$).

The values above quantify the metabolite AGN 190299. None of the parent compound, AGN 190168, was found in the high-dose samples on day 1 or 91. Other metabolites were either not quantified or the data were not reported. On day 1, only the highest dose group had quantifiable levels of AGN 190299. For this high-dose group, the day 91:day 1 C_{max} and AUC were 22 and 21, respectively. The structure of AGN 190299 is provided on the metabolic pathway presented on the following page.

Chronic (6 months or greater) Studies:

Oral:

26-week dietary study with recovery in rats; Study no. 1643-9/94 /21-931051;

Animal Strain: Crl:CD(SD)BR rats

No. of Animals: 15/sex/group; 5/sex/group retained for a 9-week recovery period

Route: dietary

Duration: daily dosing for 6 months

Study Design & Dose Levels:

Group	Dose level mg/kg/day
1-Control	0
2-Low	0.025
3-Mid	0.050
4-High	0.250

Methods: Rats were given AGN 190168 for 26 weeks in the diet. For 10/sex/group, hematology, biochemistry and urinalysis were performed at Week 25 and in the recovery animals during week 4 of recovery, and in some males, week 8 of recovery. Toxicokinetic samples were collected in week 26 (5/sex/group), and in weeks 8 and 10 of the recovery period.

Results:

Mortality: One mid-dose male was found dead in week 15.

Body Weights: High-dose male and female mean values were slightly below control values (8-15%) throughout dosing; high-dose recovery animals also were below control mean values (15%).

Food and Water Consumption: Food consumption was generally similar among groups. High-

dose males had slightly increased water consumption values, and these continued through week 5 of the recovery period.

Hematology and Biochemistry: At week 25, high dose females had significantly decreased MCV; and high dose males had significantly increased packed cell volume, hemoglobin, red blood cell count, and thrombotest values; although all differences were less than 5%. All treated animals had decreased neutrophil values (up to 30%), although the differences were statistically significant in females only. Protein values (total and albumin) were decreased in all treated animals (up to 18%), and glucose values were increased (up to 13%) at 25 weeks. Significant decreases were also noted in calcium (up to 8%) and cholesterol (up to 54%) values in treated animals. Animals in week 4 of recovery had increased platelet counts (females only, up to 12%). Week 4 recovery animals also had decreased cholesterol values in high dose males (35%).

Urinalysis: Values were comparable between groups, except for protein values. Treated females at week 25 had decreased values (up to 27%). Animals in the fourth week of recovery, however, had increased protein values (males, up to 70%) and decreased protein values (up to 33%). The importance of the urinalysis differences is thus unclear.

Ophthalmic Observations: No compound-related differences were noted.

Organ Weights: Mean thyroid values (up to 17%) and lung values (up to 18%) were increased in all treated animals compared to control values. High-dose males also had significantly increased adrenal weight values (17%).

Histopathology: Treatment related changes included an increased incidence of centrilobular fat deposition in high-dose males and increased periportal fat deposition in high-dose females. The liver changes were not noted in the recovery animals. High-dose males had an increased incidence of myocardial fibrosis (papillary muscle). In the recovery group, however, the finding was noted in both control and high-dose males, and does not appear to be treatment related.

Toxicokinetics: Mean blood levels ($n = 4-5$) following 26 weeks of dietary administration were 0.0508 ± 0.354 , 0.700 ± 0.0342 , or 1.8 ± 0.36 ng/ml for the low, mid, and high dose animal, respectively. Following 8-10 weeks of recovery, AGN 190299 was not detectable in any group. (Assay sensitivity was not specified.)

6-month toxicity study in monkeys; Study no. 1643-
4/93

985-120;

Animal Strain: Cynomolgus monkeys (*Macaca fascicularis*)

No. of Animals: 6/sex/group

Route: Nasal gastric intubation (equivalent to the oral route)

Duration: daily dosing for 6 months

Study Design & Dose Levels:

Group	Dose level mg/kg/day	Dose volume mg/ml
1-Control	0	0
2-Low	0.05	0.025
3-Mid	0.125	0.0625
4-High	0.5 (dose decreased to 0.25 after week 10)	0.25 (decreased to 0.125 after week 10)

Methods: Monkeys were dosed daily by nasal gastric intubation. The study was originally intended to be a 52-week study, but due to systemic toxicity noted especially in the high-dose group, the study was reduced to 6 months. Two animals per group were allowed an additional 8-week recovery period before necropsy.

Results:

Mortality: Two high-dose monkeys were euthanized prior to the 6-month sacrifice.

Clinical Observations: This study is somewhat unusual in that clinical observations (supported by histopathology at necropsy) gave the best indication of the toxicity associated with this compound. The submitted report summarized the findings quite well (volume 4, page 24): "The most remarkable, treatment-related clinical signs noted...were hunched posture, limited use of hind limbs with muscular atrophy and contraction of the tendons, hypoactivity, sores around the mouth and lips, slight to moderate tremors,...slight to severe kyphosis...in the high-dose animals,... with 3 animals showing limited rotational ability of the head. These conditions developed gradually during the course of the study. When the dose was lowered,...

there was no obvious improvement noted. Likewise, there was no noticeable improvement in the high-dose animals during the approximately 2-month recover period...A thorough physical examination by a staff veterinarian on anesthetized animals during Week 19 revealed slight to severe spinal rigidity and/or kyphosis in all the high-dose animals, several animals also exhibiting stiff coxofemoral joints and seborrheic dermatitis. In the low- and mid-dose groups, several animals showed some indication of spinal stiffness during this examination, and slight kyphosis was also noted in three mid-dose animals by the attending technician at the end of the treatment period. Clinically however, the low- and mid-dose animals appeared quite normal..."

Body Weights: In the high-dose groups, female body weights began to lag in the fifth week of the study and remained below control values until the end of the study; the difference was statistically significant in weeks 6, and 10 to 27. Males followed a similar pattern; statistically significant differences were noted in weeks 9 to 27. Following week 27, 2 monkeys/group were in an 8-week non-dosed recovery period. High-dose animal body weights continued to lag through the recovery period. Body weight changes also were lower in high-dose animals, although the differences were generally not significant. Mid- and low- dose animal body weights were comparable throughout the study.

Hematology (13 parameters): Occasional and transient statistically significant differences between treated and control groups were noted, although none can be considered treatment related. Surprisingly, none of the high-dose monkeys had any statistically significant differences when compared to control values.

Clinical Chemistry (15 parameters): In high-dose females, total cholesterol and albumin was significantly decreased in weeks 9, 13, and 26. Potassium was significantly increased in high-dose females in week 13, and in high-dose males and in mid- and high-dose females in week 26. All of the differences, however, were not significant in the two recovery animals assayed in week 34. Other statistically significant differences were transient and did not appear in a dose-related manner.

Ophthalmic Observations: No treatment-related findings were noted throughout the study.

Blood Pressure: Blood pressure evaluations, including mean arterial pressure, heart rate, systolic pressure, and diastolic pressure, was comparable among all groups throughout the study.

Electrocardiograms: No treatment-related changes were noted.

Organ Weights: Adrenal gland weights in males and females declined in a dose-dependant manner; mid- and high-dose adrenal weights were statistically less than control values. (Adrenal weights were normal in recovery animals.) Other changes in organ weights were sporadic and did not appear to be treatment related.

Gross Necropsy Observations: In the high-dose groups, four animals were noted with deformed hips and one high-dose male had deformed shoulder joints. All other findings, which were quite few in number, were considered incidental and not related to treatment.

Histopathology: Treatment-related changes were noted in the mid-and/or high-dose group included the femur, rib, vertebrae, sternum, and the hip. Lesions included:
femur - premature complete closure, disruption, and premature partial closure of epiphyseal growth plate, reduced trabeculae, erosion or fibrosis of articular cartilage and chronic inflammation;
rib - minimal to severe reduction in the calcified cartilage of the costochondral joint;
sternum - disruption of the intersternal cartilage and ankylosis; and vertebrae - absence or disruption of articular cartilage and ankylosis; and
hip - chronic inflammation with or without erosion of articular cartilage.
Several low-dose females had epiphyseal growth plate changes in the femur, although their relationship to treatment is unclear. Other histopathological findings were considered spontaneous and not related to treatment.

Dermal:

6-Month Dermal Toxicity Study in Rats; Study no. 1643-1725-2; Allergan, Irvine, CA; 5/90

Animal Strain: Sprague-Dawley albino rats

No. of Animals: 25/sex/group

Route: Topical Dermal

Study Design & Dose Levels: Animals were administered 0.05 ml twice/day for 6 months.

Group	Test Material
1 - control	non-treated
2 - control	placebo gel
3 - low dose	0.01% AGN
4 - mid dose	0.05% AGN
5 - high dose	0.1% AGN

Methods: Test materials were applied to the shaved backs of animals. Non-treated controls

were shaved but not treated. Three scheduled sacrifices were performed: at 3 months (5 animals/sex/group), at 6 months (15 animals/sex/group), and at 6 months plus a 23-day recovery period (5 animals/sex/group).

Results:

Mortality: Two control males and one high-dose male were found dead, and one mid-dose male was euthanized in a moribund condition; all other animals survived to scheduled euthanasia.

Dermal Observations: Erythema, ranging from very slight in placebo animals to severe in high-dose animals, was noted over the course of the study. Animals also experienced edema, hardening, abrasion, flaking/scaling, and scabs in a dose- and time-dependant manner. Findings at the treatment site decreased in severity over the 23-day recovery period, although they did not disappear altogether.

Body Weights: Male body weights in the mid- and high-dose were significantly decreased when compared to control values on days 30-180. Female body weights were statistically decreased when compared to control values for the low dose on days 45, 120, and 135, and for the mid- and high-dose groups on days 15-180. In the recovery period, no statistical differences were noted.

Hematology: Values that appeared treatment-related and were significantly different from control values includes, in males: decreases in mean cell volume and monocytes; and increases in white blood cell count and neutrophils. Following the recovery period, male neutrophil percent remained significantly different from control values. In females, significant decreases were noted in hematocrit, mean cell volume, and monocytes; and increases in neutrophils, lymphocytes, and red blood cell count. Following the recovery period, female red blood cell count and mean cell volume remained significantly different from control values.

Clinical Chemistry (20 parameters): A large number of treatment-related effects were noted, although most effects were reversed following the 23-day recovery period. When compared to control values, statistically significant changes included decreased albumin values for all treated groups in males and females, decreased albumin:globulin in mid- and high-dose males and in all treated females, increased alkaline phosphatase in mid- and high-dose males and females, decreased cholesterol in low, mid, and high-dose animals, increased triglycerides in mid- and high-dose animals, and decreased calcium in mid- and high-dose animals. Following the recovery period, the only remaining significant difference was in high-dose male decreased cholesterol. In females, remaining differences were mid- and high-dose decreased albumin, increased alkaline phosphatase, and increased triglycerides; and increased A:G in all treated female dose groups.

Ophthalmic Observations: Abnormalities following slit-lamp examination were rare in

occurrence and not related to treatment.

Organ Weights: In males, statistically significant differences in absolute organ weights included mid- and/or high-dose adrenal, spleen, liver, kidneys, pituitary, and brain. These changes were not reversed during the recovery period in any organ but the brain. In females, significant differences were noted in the low- and/or mid- dose spleen, pituitary, adrenal, ovaries, brain, lungs, and spleen when compared to control values. Following the recovery period, significant differences remained only for the spleen weight. Organ-to-body weight differences mirrored the findings for the absolute organ weights, and gave further support to the organ weight differences being treatment related.

Gross Necropsy Observations: Enlarged and/or discolored adrenal glands were noted at the 91 day sacrifice (females), at the 6-month sacrifice (males and females), and following the recovery period (females). Other organs appeared normal at the gross examination.

Histopathology: The sponsor performed three histopathological evaluations: at the 3-month sacrifice, the 6-month sacrifice, and following the 23-day recovery period. In general, abnormalities, especially at the skin treatment sites, became worse between the 3 and 6 month exposure periods and moderated after the recovery period. At 6 months, both epidermal and dermal histological changes were noted in all AGN-treated animals. Epidermal changes included ulceration, pustule formation, hyperkeratosis, parakeratosis, and acanthosis. Dermal changes include inflammation and fibrosis. The frequency and intensity of these findings increased with dose level. Systemic changes thought to be treatment related at the six-month sacrifice included the femoral bone, adrenal glands, and liver.

Following the 23-day recovery period, histological evaluation revealed that the skin treatment sites had undergone "considerable resolution," although abnormalities were noted in all treatment groups. The pathologist stated that "Continued resolution would be expected with the further passage of time." Similarly, the adrenal abnormalities and hepatic/periportal lipidosis were still somewhat abnormal, although the degree had lessened and the pathologist expected eventual resolution. No residual bone lesions were noted in the end-of-recovery period - sacrifice.

12-month dermal toxicity study in miniswine; Study no. 1643 -440B-602-244-91;
4/94

Animal Strain: Hanford miniswine

No. of Animals: 6/sex/group, 2/sex/group were retained for a recovery period of 8 weeks

Route: Topical dermal

Study Design & Dose Levels:

Group	Test Material (%)	Test Material (g/kg/day)
1 - control	placebo	treated with 0.25 placebo gel
2 - low dose	0.025	0.050
3 - mid dose	0.05	0.125
4 - high dose	0.1	0.250

Methods: AGN 190168 was applied twice/day to the clipped intact backs of miniswine for at least 365 days. Animals were examined quarterly with physical and ophthalmologic exams, and blood samples were taken. Body weights were recorded weekly.

Results:

Mortality: No unscheduled deaths occurred during the study.

Dermal Observations: In week 2, treated animals were noted with erythema at the treated sites and the erythema appeared to increase in severity and number of animals effected over time. By day 22, however, a hard black colored scab had formed over the treatment sites and they could generally not be evaluated for the remainder of the study (occasional erythema was noted when sites could be graded). During recovery for females, the scab began to fall off of the treated site and the skin appeared to be healing. For males, the treated site remained dark and ungradable.

Body Weights: No treatment-related changes were noted in body weight values.

Hematology and Clinical Chemistry: No treatment-related changes were noted in hematology values. After 13, 19, 26, 39, or 52 weeks of treatment, increases in total protein and globulin in treated animal, and decreased were noted in albumin values. At recovery, however, these values were similar to control animals.

Ophthalmic Observations: No treatment-related differences were noted.

Organ Weights: No treatment-related differences were noted.

Gross Necropsy Observations: Outside of the treated skin site, no treatment related differences were noted.

Histopathology: Treated skin was noted with microscopic signs of irritation, including acanthosis, dermal inflammatory cellular infiltration, cellular surface debris, neutrophilic cellular infiltration, focal erosion, focal ulceration, and focal dermal fibrosis. No treatment-related systemic changes were noted. In the recovery animals, similar skin changes were

noted, as well as signs of healing including slight hyperkeratosis.

Pharmacokinetics: AGN 190168 and its main metabolite, AGN 190299, were below quantifiable levels (sensitivity 0.05 ng/ml) in vehicle control animals. In treated animals, values ranged from ng/ml for the low and high-dose animals.

Reproduction & Teratology Studies :

Dermal range-finding teratology study in rats; Study no. 1643 3202.4A; 5/92

Sprague Dawley rats (6/group) were dermally administered AGN 190168 at dosage levels of 50, 125, 250 and 500 µg/kg/day (dosage volume 0.5 ml/kg, except for the high dose which received 1.0 ml/kg). Animals were collared and treated on their shaved backs during gestation days 6-17. All females survived to gestation day 20 and no clinical signs of toxicity were noted during the study. Body weights were slightly decreased in the treated animals. Skin irritation (erythema, scabs, hair loss, eschar) was noted on all treated animals and severity increased with dose. No treatment-related differences were noted among the control and treated groups in the number of viable fetuses, early resorptions, sex ratios, and fetal weights. High-dose females had fewer corpora lutea, implantation sites, and viable fetuses. At the high-dose, one fetus had a filamentous tail and anal atresia and one fetus had head anomalies and brachydactyly.

Comment: The sponsor (via their contract laboratory report) misrepresented the results from this study in the study summary. The findings in the high-dose female (except for the malformation) were not mentioned and the body weight changes were not accurately represented.

Range-finding developmental toxicity study in rats; Study no. 1643 3202.11; 8/94

Sprague Dawley rats (6/group, plus 12/group for toxicokinetics) were orally dosed with placebo, 0.05, 0.25, 1.0, or 2.5 mg/kg/day on gestation days 6-17. One high-dose and three high-dose females died or were sacrificed on study. High-dose body weights were below control values on day 20 (11%), and body weight gains were also depressed in the high-dose animals. On day 20, high dose females were noted with increased leucocyte values (11%), AST (18%), ALT (40%), ALK (22%), phosphorus (20%), and cholesterol (16%); and decreases in platelet (15%) and glucose (14%) values when compared to mean control values. At cesarean section, high-dose and to a lesser extent, mid-dose (1.0 mg/kg), females had decreased corpora lutea, implantation sites, viable fetuses, and fetal weights; and an increase in early resorptions and post-implantation loss. In the satellite groups (12/group), blood samples for toxicokinetics were collected on gestation day 17 at 0.5 to 24 hours. Data are

summarized below.

Dose ($\mu\text{g/kg}$)	C _{max} (ng/ml)	T _{max} (hr)	AUC 0-24 ($\text{ng}\cdot\text{h/ml}$)	t _{1/2} (hr)
0	blq	na	na	na
0.05	20.4 \pm 6.63	1.0	60.1	3.59
0.25	50.7 \pm 12.3	0.5	115	5.91
1.0	193 \pm 62	0.5	510	3.85
2.5	637 \pm 134	1.0	1200	6.97

**Dermal range-finding teratology study in rats with Retin-A; Study no. 1643- 3202.4B;
5/92**

Sprague Dawley rats (6/group) were dosed with 0, 50, 125, 250, 500 $\mu\text{g/kg/day}$ Retin-A on days 6-17 of gestation. Animals were collared and treated on their shaved backs. Erythema was noted in all Retin-A-treated animals, and increased in severity and incidence with dose. Desquamation and eschar was also noted in the mid- and high-dose animals. Body weight gains were slightly decreased in the high-dose animals. No clear treatment related differences were noted in the pregnancy parameters. One incidence of cleft palate was noted in one high-dose fetus and one incidence of anophthalmia was noted in a low-dose fetus. No other malformations were noted.

**Dermal teratology study in rats with AGN 190168 and Retin-A; Study no. 1643- 3202.1;
4/89**

Female Sprague Dawley rats (25/group) were dosed with vehicle, 125 $\mu\text{g/kg/day}$ AGN 190168, or 125 $\mu\text{g/kg/day}$ Retin-A gel. Compound was applied daily to collared females on gestation days 6-15. All groups were noted with scabs, hair loss and erythema; erythema was greatly increased in the two retinoid-treated groups compared to the vehicle control. Body weights and food consumption were comparable. Pregnancy was slightly decreased in the treated animals (92% compared to 96% pregnancy in controls). Fetal data were comparable, except for a 50% increase in pre-implantation loss in the Retin-A group compared to the control and AGN 190168 groups. Malformations were minimal and were comparable between groups.

Dermal peri- and postnatal study in rats, Study no. 1643-3202.10;
8/93

Female Sprague Dawley rats (25/group) were topically dosed with 25, 50, or 125 $\mu\text{g/kg/day}$ AGN 190168 on gestation day 16 through lactation day 20. F_0 females were allowed to deliver and rear the F_1 generation. F_1 pups (20/sex/group) were mated at 12 weeks, allowed to deliver and rear their offspring until lactation day 21. In the F_0 generation, one high-dose female was found dead during parturition. Slight erythema and eschar was noted in all treated groups; incidence increased with dose. Skin thickening was noted in the high-dose animals. Body weights and food consumption were not affected by drug treatment. A slight decrease in the number of pups retrieved on lactation day 6 (2 days after culling) was noted for the high-dose females (89% compared to 95% in the control group). This difference continued to lactation day 21, where the high-dose group had statistically fewer pups than the control group (99.5% vs. 94.7%). The high-dose group also had an increased number of presumed cannibalized pups (9 vs 2 in the control group) and an increased number of found dead pups (31 vs 16 in the control group). No behavioral differences were noted among the F_1 pups. The F_1 maturation body weights were comparable among groups, and no body weight differences were noted during lactation in the F_1 dams. No treatment-related effects were noted in the copulation and fertility indices, pregnancy rates, or necropsy. F_2 pup viability and body weights were comparable throughout lactation, and no treatment-related effects were noted at necropsy.

Developmental toxicity study in rats; Study no. 1643-3202.12;
12/94

Female Sprague Dawley rats (45/group; 5/group for toxicokinetic analysis) were dosed orally with AGN 190168 (0.0, 0.05, 0.25, or 1.0 mg/kg) while pregnant during organogenesis. Females euthanized prior to scheduled death included 8 high-dose, one mid-dose, and one control female(s). Body weights were significantly ($<10\%$) decreased for high-dose females after day 9, and occasionally decreased in the mid-dose females; decreased food consumption was also noted in the two groups. High-dose females had significantly decreased fetal weights (16%) for their litters. A significantly large number of malformations were noted in the high dose fetuses and litters, including expected retinoid malformations of cleft palate and skull anomalies; overall there were 2, 0, 8, and 309 malformations in groups 1-4, respectively. On lactation day 0, the high-dose had significantly more (80%) dead pups than the control group; the number of pups again dropped in the high dose group by day 7 (3 days after day 4 culling) to 95.3, compared to 100% in the control group. High-dose pup observations during lactation included cephalocele, exencephaly, facial papilla anomaly, pinna anomaly and cleft palate. High-dose pups also had lower body weights, decreased righting response, cliff aversion, delayed eye opening, startle and auditory response, and delayed testes descent and vaginal opening, and open-field descent. Occasional developmental delays were also noted in the mid-dose pups. During maturation of the F_1 pups, male and female high-dose body weights were significantly below control values (25%). High-dose females had significantly lower gestation

weight values (15-20%), decreased implantation scars, litter sized, and no. of pups alive on day 1 and on day 4 prior to culling. At necropsy, no treatment -related observations were noted in the F₂ pups (all groups.)

Dermal teratology study and 2-generation reproduction study in rats with AGN 190168 and Retin-A; Study no. 1643 -3202.5; 11/91

Number of Animals/Group: 40/group

Animal Strain: Sprague Dawley Crl:CD*BR VAF/Plus* rats

Route of Administration: Topical dermal

Dose levels/Study Design:

Group	Test Material	Dose Level (µg/kg)	Dose Conc. (%)	Dose Conc. (µg/ml)	Dose Volume (ml/kg)
1-control	vehicle	0	0.00	0.0	0.5
2-low	AGN 190168	50	0.01	100	0.5
3-mid	AGN 190168	125	0.25	250	0.5
4-high	AGN 190168	250	0.50	500	0.5
1 - low	Retin-A	50	0.01	100	0.5
2 - mid	Retin-A	125	0.25	250	0.5
3 - high	Retin-A	250	0.50	500	0.5

Methods: Treatments were applied on the clipped dorsal trunk of females. At the F₀ cesarian section, 25 dams were sacrificed on Day 20 of gestation. Females were examined and reproductive/teratogenic parameters were recorded. The remaining 15 females were allowed to deliver (if pregnant) and the F₁ litters were evaluated for survival and development. Fifteen of the F₁ pups were selected as parental animals and mated at 12 weeks; the F₂ litters were evaluated and necropsied on day 21. All litters were culled on lactation day 4.

Results (Discussed in order of generation):

Mortality: No unscheduled deaths occurred.

Observations: There were no treatment-related observations.

Dermal Evaluation: Slight to moderate erythema and slight desquamation were noted in all of the retinoid-treated groups. Irritation (severe erythema) progressed in a dose-dependent manner to eschar formation with subsequent exfoliation.

Body Weight Gain and Food Consumption: No differences were noted between groups during lactation. During gestation, the AGN 190168 mid- and high-dose groups had significantly decreased body weights. The AGN 190168 mid- and high-dose groups also were noted with reduced food consumption.

Gross Necropsy: No treatment related differences were noted.

Pregnancy Rate: Pregnancy rate was comparable among groups.

Cesarian Section Data: No differences were noted among the groups.

Fetal Malformations/Variations: Malformations were slight and did not appear in a dose-dependent manner. A variation (not developed renal papillae) was noted in a single pup in the mid- and high- dose of both retinoids.

Pup Viability: No meaningful differences were noted, although there was a 5% increase in dead pups in the high-dose AGN 190168 group. (Historical control value for dead pups is 4%.)

Pup Observations: No treatment-related differences were noted.

Pup Body Weight Gain and Food Consumption: The AGN 190168 groups had lower body weights in the mid- and high-dose groups; other groups were comparable.

Pup Gross Necropsy: No treatment-related differences were noted.

Pup Developmental/Emotional Parameters: Groups appeared similar.

Body Weights: In the AGN 190168 groups, lower body weights were recorded in the mid- and high-dose groups as compared to the controls. The Retin-A groups had normal gains.

Gross Necropsy: No unusual observations were made.

Pregnancy Rate: No treatment-related effects were noted.

Cesarian Section Data: No group related differences were noted.

F2: Pup Viability: A significant increase in the number of dead pups on lactation day 0 was noted for both the AGN mid dose and the Retin-A high dose. (The values were within historical control values.) All other parameters were normal for the pups.

**Dermal Fertility and Reproduction Study in Rats; Study no. 1643-3202.7,
6/93**

Number of Animals/Group: 40/sex/group

Animal Strain: Sprague Dawley Crl:CD[®]BR VAF/Plus[®] rats

Route of Administration: Topical dermal

Dose levels/Study Design: Dose levels are summarized below.

Group	Test Material	Dose Level (µg/kg)	Dose Conc. (%)	Dose Conc. (mg/ml)	Dose Volume (ml/kg)
1-control	vehicle	0	0.00	0.0	0.5
2-low	AGN 190168	25	0.01	0.1	0.25
3-mid	AGN 190168	50	0.01	0.1	0.5
4-high	AGN 190168	125	0.05	0.5	0.25

Methods: Test materials were placed daily on the clipped backs of rats. Males were dosed for 70 days prior to mating and until euthanasia. Females were dosed for 14 days prior to mating, throughout gestation and lactation, and until euthanasia.

Following treatment (70 or 14 days depending on gender), each F0 female was bred to a single male from the same treatment group. If the pair cohabitated for 10 days without breeding, females were placed with a proven male from the same treatment group. After 15 days without breeding, females were placed in a nesting cage.

The first 24 mated females were euthanized on gestation day 20. Each female was examined at gross necropsy, and the uterus was evaluated for reproductive parameters. Fetuses were

examined for external, visceral, and skeletal abnormalities.

The remaining females were transferred to nesting boxes on gestation day 19, and females remained with their offspring until lactation day 21. Offspring were designated the F1 generation. On lactation day 4, litters were randomly culled to a maximum of eight pups. Culled pups were weighed and euthanized. Pup viability was determined daily, and developmental and functional evaluations were performed during lactation. (Evaluations included pinnae detachment, surface righting, cliff aversion, eye opening, startle response, auditory response, testes descent, vaginal opening, open-field testing, and Biel water maze.) All surviving F0 females and males were euthanized and examined grossly for abnormalities. Following weaning, 20 pups/sex/group were chosen as the parental animals of the F2 generation. At 12 weeks of age, F1 females were bred to F1 males of the same treatment group. (Because of a low copulatory rate, after the initial 15-day period, an additional 5 days was scheduled for non-bred females with a proven male.) On lactation day 4, litters were culled to 8 pups. Females and their offspring remained together until lactation day 21. All surviving F1 males, females, and F2 pups were euthanized on the F2 lactation day 21. All animals were examined grossly and females were evaluated for reproductive parameters.

Results (Discussed in order of generation):

Mortality: One mid-dose male and 3, 2, 2, and 3 females in Groups 1-4, respectively, were euthanized in extremis.

Observations: One low-dose and one mid-dose male had rales or wobbly gait, respectively. Numerous scabs, skin lesions, and hair loss were noted in the females and males, especially in the treated groups. One low-dose female had a palpable mass in the right lateral thoracic area. Other observations were considered within the range of normal.

Dermal Evaluation: At the exposure site, AGN-treated males and females were noted with slight-to-moderate erythema, edema, desquamation, and eschar areas. Five high-dose females also had skin thickening.

Body Weight Gain and Food Consumption: Differences in body weights in males and females occurred sporadically and in a transient nature, and do not appear to be treatment related. Differences in food consumption values were also sporadic and not considered to be treatment-related.

Gross Necropsy: Findings occurred spontaneously and were not related to treatment.

Pregnancy Rate: The rate was 22, 21, 21, and 22 pregnancies in groups 1, 2, 3, and 4, respectively.

Fertility Parameters: Copulatory and fertility indexes, precoital interval, and gestation length

were comparable between the treated and control groups.

Reproduction Parameters: By lactation day 6, the mid- and high-dose animals had significantly fewer pups when compared to control values. No other significant differences were noted between treated and control groups.

Fetal Malformations: No significant differences were noted between treated and control groups. Total malformations were noted in 1, 5, 1, and 2 fetuses, respectively in groups 1-4. A larger number of 14th rudimentary ribs was noted in high-dose fetuses and litters, although the incidence was not statistically greater than control values.

F1: **Pup Viability:** Mean live litter size on lactation days 1-4 for the F1 pups decreased with dose, although the difference was statistically significant only in the mid-dose group. On lactation day 4, no. alive/no. pups was 210/214, 217/224, 203/215, and 184/189, for groups 1-4, respectively. After pups were culled on Day 4, two pups died by day 21 in the high-dose group; 1 pup died in the control group.

Pup Observations: During lactation, a variety of observations were made for all groups, including subcutaneous hemorrhage, fluid filled abdomen, severed tail tip, hair loss, prolapsed rectum. Overall, fewer normal findings were noted in the high-dose group: 736, 767, 742, and 670, for groups 1-4, respectively. No single finding, however, could be clearly related to treatment.

Pup Body Weight Gain and Food Consumption: F1 pup body weights were similar throughout lactation.

Pup Gross Necropsy: The number of pups found dead or sacrificed in extremis was 6, 7, 7, and 9 for groups 1-4, respectively. Although no single gross necropsy observation could be related to treatment, the number of remarkable findings increased with dose: 1, 4, 4, and 8 pups for groups 1-4, respectively.

Pup Developmental/Emotional Parameters: No treatment-related differences were noted in emotional development tests of the F1 pups.

Clinical Observations during F1 Maturation (Weeks 5-23): One high-male was euthanized in extremis; the remaining 20 males/group survived to scheduled euthanasia. In females, of a possible 20/group, 12, 15, 16, 15 survived to scheduled euthanasia in groups 1-4, respectively. Clinical observations for the males and females were within the range of normal for this strain and age of animal.

Body Weights: During maturation, body weights and gains lagged slightly for high-dose males, although the difference was not statistically significant. During maturation, gestation, and lactation, female body weights and body weight gains were comparable between the

control and treated groups.

Gross Necropsy: One high-dose male and one mid-dose female were euthanized in extremis. No treatment related findings were noted in the scheduled or non-scheduled euthanized animals.

Pregnancy Rate: Total number of gravid females (and females that delivered) was 12, 15, 16, 15 for Groups 1-4, respectively.

Fertility Parameters: No treatment related differences were noted in copulation, fertility, precoital interval, or gestation length.

Reproduction Parameters: No significant differences were noted between treated and control groups.

F2: Pup Viability: No treatment-related differences were noted.

Pup Observations: No treatment-related differences were noted.

Pup Body Weight Gain and Food Consumption: No treatment-related differences were noted.

Pup Gross Necropsy: No treatment-related differences were noted.

**Dermal range-finding teratology study in rabbits; Study no. 1643- 3202.8;
7/94**

New Zealand white rabbits (5/group) were dosed dermally on their shaved backs with 0, 50, 125, 250, or 500 $\mu\text{g/kg}$ AGN 190168 on days 6-18 of gestation. The rabbits were restrained with Elizabethan collars for 6 or more hours after dosing. Animals were sacrificed on day 29 of gestation and fetuses were evaluated. Dose-related erythema and desquamation were observed in all groups. Similar body weights were noted across groups. One high-dose female aborted on gestation day 21. Pregnancy rate was reduced by AGN 190168 treatment; the rate dropped from 100% to 60-80% for the three highest doses. A reduction in the number of corpora lutea and implantation sites was noted in the two highest dose groups. No external malformations or variations were noted.

**Dermal teratology study in rabbits; Study no. 1643- 3202.9;
4/92**

New Zealand white rabbits (20/group) were topically dosed with AGN 190168 (0, 50, 125, or 250 $\mu\text{g/kg}$) on gestation days 6-18. Test articles were applied to the dorsal clipped backs, and rabbits were affixed with Elizabethan collars for a minimum of 6 hrs; excess test article was then wiped from the skin. Animals were sacrificed on Day 29. (On several occasions, rabbits

were found outside their collars, which would allow opportunity for oral ingestion of the test articles.) No females died on study. Erythema was noted across the AGN 190168-treated groups; eschar was noted with increasing incidence in the highest dosed groups, and a single animal in the high-dose groups was noted with fissuring. Body weight values and food consumption were similar across groups. Pregnancy and abortion rates were not effected by drug treatment. No treatment-related differences were noted in cesarean section parameters. Malformations of concern included single incidences of spina bifida, hydrocephaly, and heart anomaly in the high dose group,

**Range-finding developmental toxicity study in rabbits; Study no. 1643- 3202.13;
; 7/94**

New Zealand white rabbits (8/group; 3/group for TK studies) were dosed orally with vehicle, 0.05, 0.25, 1.0, or 2.5 mg/kg day AGN 190168 during organogenesis (gestation days 6-18). At all treatment levels except for 0.05 mg/kg, a high number of abortions (40-60%) were noted. Platelet count, AST, ALT, alkaline phosphatase, glucose, cholesterol were increased in the three highest dose groups (up to 50%); and phosphorus and triglyceride values were decreased. In cesarean section data, the three highest dose groups had decreased corpora lutea, implantation sites, viable fetuses (0 in the two highest-dose groups); and increased early resorptions and post-implantation loss. Fetal loss was so high that no malformations of variations were noted in any of the treated groups.

Toxicokinetics sampling on day 18 (last dose) indicated that the AGN 190299 (main metabolite) C_{max} (μg/ml) was 0.0560 ± 0.0054 to 2.35 ± 0.78, and T_{max} (hr) was 5.55 to 1.00 for the low and high doses, respectively. In general, C_{max} increased proportionally to dose. Of some concern is that low levels of AGN 190168 and its metabolite AGN 190299 were found in the placebo animals. The sponsor could not explain this apparent serious protocol deviation.

**Developmental Toxicity Study in rabbits; Study no. 1543- 3202.14;
9/94**

AGN 190168 was administered orally to New Zealand white rabbits (23/group plus 3/group for TK studies) at doses of 0.025, 0.050, and 0.200 mg/kg/day. No treatment related changes were noted in the low- and mid-dose groups. In the high-dose, there was an increase in the number of early resorptions and post-implantation loss. An increase in malformations was also noted in the high-dose group, including pinnae anomalies, cleft palate, spina bifida, heart anomalies, skull anomalies, hyoid anomalies, and tympanic ring anomalies.

Mutagenicity:

Ames/Salmonella Mutagenicity Assay; Study no. 1643- 301- -004-89;

11/89

AGN 190168 was evaluated in the Ames/Salmonella plate incorporation assay to determine the ability of the drug to induce reverse mutations in the presence and absence of S-9 to mimic metabolism. AGN 190168 was tested at doses of 50, 167, 500, 1670, 3330, and 5000 $\mu\text{g}/\text{plate}$. At concentrations greater than 167 $\mu\text{g}/\text{plate}$, the test article precipitated in solution. The test article was negative for induced mutations both with and without S-9.

In vitro chromosome aberration analysis in human lymphocytes; Study no. 1643-324-001-90; 2/91

The potential of AGN 190168 to induce structural chromosomal aberrations was evaluated in human lymphocytes in culture at three phases of the cell cycle (G0/G1, G2, and S phases). Two positive controls, Mitomycin-C (a direct acting mutagen) and cyclophosphamide (a proclastogen requiring metabolic activation), and a vehicle (DMSO) control were included in the test. AGN 190168, after testing for cytotoxicity to set dose levels, was evaluated at 40, 175, and 400 $\mu\text{g}/\text{ml}$ without S-9, and 1-20 $\mu\text{g}/\text{ml}$ with S-9. No statistically significant increases in the proportion of aberrant metaphase was noted at any AGN 190168 dose or time interval.

E. Coli liquid pre-incubation assay; Study no. 1643-301-001-93; 7/94

AGN 190168 was evaluated in E. coli tester strain WP2 uvrA to determine the potential of the drug to induce reverse mutations at the tryptophan locus with and without S9. AGN 190168 was evaluated at 5-1000 $\mu\text{g}/\text{plate}$, although the test article was not completely soluble above 167 $\mu\text{g}/\text{plate}$. Revertant frequencies for all doses were approximately equal or less than the negative controls.

CHO/HPRT Mammalian cell forward gene mutation assay: Study no. 1643-314-002-94; 1/95

AGN 190168 was evaluated in the CHO/HPRT mammalian cell forward gene mutation assay to determine the potential of the drug to induce mutations at the hypoxanthine-guanine phosphoribosyl transferase (HPRT) locus in cultured Chinese hamster ovary (CHO) cells. AGN 190168 was evaluated in duplicate cultures at 0.5-500 $\mu\text{g}/\text{ml}$ with S-9 and 0.5-5000 $\mu\text{g}/\text{ml}$ without S-9; precipitate was noted at concentrations above 16.7 $\mu\text{g}/\text{ml}$ and extreme cytotoxicity was noted at concentrations above 167 $\mu\text{g}/\text{ml}$. Cultures treated with AGN 190168 had no statistically significant or dose-dependent increases in mutation frequencies.

In vivo micronucleus test in mouse bone marrow erythropoietic cells; Study no. 1643-309-003-93; 9/94

The potential of AGN 190168 to induce micronuclei in polychromatic erythrocytes (PCEs) in mouse bone marrow was evaluated at doses of 0.1-100 mg/kg. Mice received a single IP injection and were sacrificed 24, 48, or 72 hours post dosing. A negative control (corn oil) and a positive control (triethylenemelamine) group were included. Bone marrow from mouse femur was collected and evaluated for micronucleated PCEs and micronucleated normochromatic erythrocytes (NCE). A statistically significant increase in MPCE frequency was observed for females treated at a dose of 1 mg/kg at 24 hours. However, no other increases were noted at two higher dose levels, and thus the increase was not considered meaningful. Occasional increases and decreases were noted in the PCE/NCE ratios, but the occurrences were variable and did not appear to be treatment related. The positive control gave the expected significant decrease in PCE/NCE ratios. Overall, AGN 190168 was considered to be negative (non-clastogenic) in this assay. Blood levels at 2 and 4 hours post dosing were determined in satellite groups; samples indicated a dose-related systemic exposure to AGN 190168.

Carcinogenicity and Dose-Finding Studies:

Dose-selection photocarcinogenicity study; Study no. 1643- 1801-001
6/93

Number of Animals: 3/sex/group

Animal Strain: Albino hairless mice [CrI:SKH1 9hr/hr]BR]

Study Design and Dose Levels: Animals were treated with 100 μ l of test material.

Group	Test Material
1	AGN placebo
2	0.1% AGN
3	0.01% AGN
4	0.001% AGN
5	0.0001% AGN

Route of Administration: Topical Dermal

Length of Exposure: Single exposure plus UVR light (sunlight simulation)

Methods: In the first study (Study I), animals were lightly anesthetized with chloral hydrate, test articles were applied topically, and selected sites were irradiated with UVR (0 to 2.7 times

MedD; MedD = a UVR dose adequate to cause a barely perceptible response in the skin of hairless mice). At 24, 48, and 72 hours after irradiation, animals were examined for inflammation, and graded for the protectiveness factor (PF) of the test material.

In a second study (Study II) mice were treated with repeated doses (5 days/week for 8 weeks) of the test materials (0 to 1.0 mg/ml) with UVR exposure to determine the most appropriate concentrations for a full blown photocarcinogenicity study.

Results:

Study I: AGN at all treatment levels did not elicit UVR responses indicative of phototoxicity or photoprotection.

Study II: Treatment with 1.0 mg/ml caused severe cutaneous inflammation; 0.1 mg/ml caused mild to moderate cutaneous inflammation, and 0.01-0.001 mg/ml caused mild cutaneous inflammation. Irritation seemed to occur more in males than in females.

Based on the above two studies, recommended dose levels were 0.1 and 0.01 mg/ml, which are equivalent to the concentrations of tretinoin that enhance photocarcinogenesis.

12-month photocarcinogenicity study in hairless mice; Study no. 1643 1801-001;
2/95

Animal Strain: Albino Hairless Mice CrlArg:SKH1 (hr/hr)BR

No. of Animals: 36/sex/group

Route: Dermal

Duration: Approximately 52 weeks