CENTER FOR DRUG EVALUATION AND RESEARCH

APPLICATION NUMBER:

NDA 21-753

PHARMACOLOGY REVIEW

DEPARTMENT OF HEALTH AND HUMAN SERVICES
PUBLIC HEALTH SERVICE
FOOD AND DRUG ADMINISTRATION
CENTER FOR DRUG EVALUATION AND RESEARCH

PHARMACOLOGY/TOXICOLOGY REVIEW AND EVALUATION

NDA NUMBER: **21-753**

SERIAL NUMBER: **N000**

DATE RECEIVED BY CENTER: **01/04/04**

PRODUCT: **DIFFERIN^{R} XP^{TM}** (adapalene gel, 0.3%) **Gel**

INTENDED CLINICAL POPULATION: Subjects above 12 years of age

SPONSOR: Galderma Laboratories, L.P.

14501 N. Freeway, Fort Worth, TX 76177

DOCUMENTS REVIEWED: Vol. 1.1, 1.8-1.35

REVIEW DIVISION: Division of Dermal and Dental Drug Products (HFD-540)

PHARM/TOX REVIEWER: Kumar D. Mainigi

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DIVISION DIRECTOR: Jonathan Wilkin

PROJECT MANAGER: Millie Wright

Date of review submission to Division File System (DFS):

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EXECUTIVE SUMMARY

I. Recommendations

A. Recommendation on approvability: Approvable

B. Recommendation for non-clinical studies: None

C. Recommendations on labeling: The draft submitted by the sponsor is accepted after some modifications.

II. Summary of non-clinical findings

A. Brief overview of non-clinical findings:

Irrespective of the formulation and or drug concentration, the average topical absorption of adapalene in most species including man did not exceed 5 percent. The drug and its metabolites are mainly distributed in tissues and organs involved in excretion. The low drug accumulation on repeated applications indicated fast metabolism. Adapalene is extensively metabolized in animals and humans, however, its metabolic pathways and metabolites have not been completely characterized.

In rats, the placenta acted as a partial barrier to drug and its metabolites during organogenesis and thereafter. Adapalene is also secreted in the milk of rats.

No systemic toxicity was observed in sub-chronic and chronic topical studies conducted at the maximum feasible dose of 2g of 0.3% adapalene gel/kg/day. The dose-related dermal lesions such as scabs, acanthosis, epidermal hyperplasia, and superficial inflammation on the application sites, were mild and reversible in nature.

In the oral studies (1-500mg adapalene/kg/day), loss of hairs, body weight, spontaneous long bone fractures, and skeletal resorption resembled hypervitaminosis A syndrome.

Adapalene did not exhibit mutagenic or genotoxic effects *in vivo* (mouse micronucleus test) and *in vitro* (Ames test, Chinese hamster ovary cell assay, and mouse lymphoma TK assay) studies.

In the mouse dermal carcinogenicity study, no drug-related neoplastic lesions were observed. In the rat oral carcinogenicity study, the high-dose males (1.5mg/kg/day) exhibited a significant ($p<0.05$) incidence of benign pheochromocytoma of the adrenals. The combined number of benign and malignant pheochromocytoma, and pancreatic islet cell tumors in drug-

treated males indicated a higher incidence. According to the sponsor, the high incidence of pheochromocytoma is a characterisitic of compounds acting like retinoids. Moreover, there are many morphological and biochemical differences between the adrenal glands of the rat and man. In addition, the incidence of pheochromocytoma in man is very low (0.005 to 0.09%). A high incidence of carcinomas and adenomas of thyroid was also observed in the drug treated females.

No photocarcinogenicity study was conducted.

In the oral studies (1.5-20mg/kg/day), no effects on reproductive performance, fertility, litter size, growth, development, weaning, and subsequent reproductive performance of the offspring were observed.

In the dermal teratology studies (6mg/kg/day) in rats and rabbits, no teratologic changes were observed. However, in the oral rat and rabbit studies (5, 25, and 60mg/kg/day), significant teratologic changes (skeletal and visceral malformations) were recorded at 25mg/kg/day and higher dose levels.

In the prenatal and postnatal development studies (0.15, 1.5, and 15mg/kg/day), the highest dose of adapalene had no effect on the evaluated litter parameters (development after weaning, mating and fertility) of F_0 and F_1 generations, and on F_2 fetuses. Since adapalene was excreted in the milk, it is inferred that the pups were exposed both *in utero* and during lactation.

Adapalene gel at 0.3% strength was well tolerated. It did not cause any phototoxicity or photo-allergenicity in guinea pigs. Adapalene also did not induce any sensitization in guinea pigs.

B. Pharmacologic activity:

Adapalene in addition to displaying typical retinoid effects (e.g. normalization of the maturation of follicular epithelium) also exhibits some anti-inflammatory properties. Like retinoic acid, adapalene also activates nuclear receptors and inhibits transglutaminase I, an enzyme involved in the terminal differentiation of keratinocytes. The drug also expressed comedolytic activity in Rhino mouse containing a high density of spontaneous "comedones".

Animal studies have indicated that adapalene is a potent modulator of cellular differentiation, keratinization, and inflammatory processes, all of which represent important features in the pathology of acne vulgaris. Thus, topical adapalene normalized the differentiation of follicular epithelial cells resulting in decreased microcomedone formation. It also inhibited the lipoxidation of arachidonic acid to inflammatory mediators.

Adapalene gel was also efficient in repairing the signs of UVB-induced photodamage (acanthosis, inflammation, elastosis etc.) on the skin of hairless mice.

C. Non-clinical safety issues relevant to clinical use: None

2.6 PHARMACOLOGY/TOXICOLOGY REVIEW

2.6.1 INTRODUCTION AND DRUG HISTORY: Adapalene drug products have been approved in several European, [ ] Latin and North American countries and Australia for the treatment of *acne vulgaris*. Two formulations of adapalene, DIFFERIN™ (adapalene solution) Solution 0.1% (NDA 20-338), and DIFFERIN™ (adapalene gel) Gel 0.1% (NDA 20-380) have been marketed in the United States since August 1996. Third preparation DIFFERIN™ (adapalene) Cream 0.1% (NDA 20-748) was approved in May 2000.

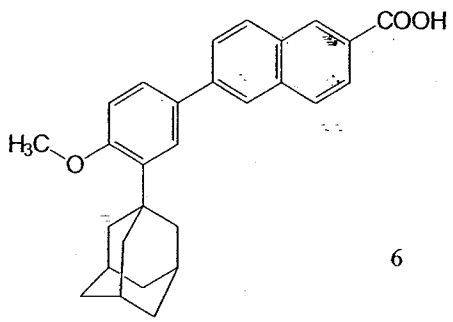
NDA number: **21-753**
Review number: 01
Sequence number/date/type of submission: N000/01-04-2004/original
Information to sponsor: No
Sponsor and/or agent: Galderma Laboratories, L.P.
Fort Worth, TX 76177
Manufacturer for drug substance: [ ]

Reviewer name: Kumar D. Mainigi
Division name: Dermal and Dental Drug Products
HFD #: HFD-540
Review completion date:

Drug:

Trade name: DIFFERIN® XP™ Gel
Generic name:
Code name: Galderma CD 271
Chemical names: 1) 2-Naphthalenecarboxylic acid, 6-(4-methoxy-3-tricyclo[3.3.1.1^{3,7}]dec-1-ylphenyl-;
2) 6-[3-(1-Adamantyl)-4-methoxyphenyl]-2-naphthoic acid.

CAS registry number: 106685-40-9
Molecular formula/molecular weight: $C_{28}H_{28}O_3$/412.5[ ]
Structure:



Relevant INDs/NDAs/DMFs:
INDs.. 31, 997 (Lotion), [redacted]
33, 540 (Gel), [redacted]
38, 508 (Cream), Owen/Galderma Laboratories, Inc., Fort Worth,
NDAs..20-338 DIFFERIN[R] (solution) Solution 1% Approved May 31, 1996
20-380 DIFFERIN[R] (adapalene gel) Gel 0.1% Approved May 31, 1996
20-748 DIFFERIN[R] (adapalene) Cream 0.1% Approved May 26, 2000

Drug class: Naphthoic acid class of anti-acne agent
Intended clinical population: Subjects above 12 years

Clinical formulation:

Active ingredient	% w/w
Adapalene	[redacted]
Excipients	
Carbomer[1] 940, NF	
Edetate disodium, USP	
Methylparaben, NF	
Poloxamer 124, NF	
Propylene glycol, USP	
Sodium hydroxide, NF	
[redacted]	
Hydrochloric acid[2], NF	
Purified water, USP	

[1] [redacted]
[2]For pH adjustment if required

Route of administration: Topical

Disclaimer: Tabular and graphical information are constructed by the reviewer unless cited otherwise.

[For (b)(2) applications: N/A

Studies reviewed within this submission:

Pharmacology

1. Affinity for retinoic acid nuclear receptors
2. Effect on retinoic acid receptors

3. Affinity to cytosolic retinoic acid binding proteins
4. Effect on transactivation of nuclear receptors
5. Effect on epidermal transglutaminase
6. Evaluation of anti-inflammatory activity on arachidonic acid-induced edema
7. Evaluation of anti-inflammatory activity on TPA-induced edema
8. Effect on UVB-induced photodamage

Pharmacokinetics

1. Ten-days topical pharmacokinetic study in female rats
2. PK studies in rats following single intravenous, oral, and topical doses
3. Fourteen-days dermal pharmacokinetic study in rabbits
4. PK studies in dogs following single intravenous, oral, and topical doses
5. Whole body autoradiography in rats
6. *In vitro* metabolism studies in rats and humans
7. Metabolism in rat hepatic microsomes
8. Effect on hepatic enzymes
9. Excretion studies in rats
10. Milk secretion studies in rats

Toxicology

Single-dose toxicity

1. Acute dermal study in rats
2. A primary skin irritation assay in rats

Repeat-dose toxicity

1. Four-weeks dermal study in rats
2. Twenty-six-weeks dermal study in rats
3. Twenty-six-weeks oral and dermal studies in dogs
4. Twenty-six-weeks oral study in dogs

Studies <u>not</u> reviewed within this submission:

Pharmacology

1. Comedolytic efficacy of adapalene gel 0.1% (European and Japanese formulations) in mice.
2. Comedolytic efficacy of adapalene gel in mice (Japanese formulation).

Safety Pharmacology:

3. Irwin test in mice (oral)
4. Locomotor activity in mice (oral)
5. Hexobarbital sleeping time in mice (oral)
6. Anticonvulsant activity in mice (oral)
7. Proconvulsant activity in mice (oral)
8. Analgesic activity in mice (oral)
9. Body temperature in rats (oral)
10. Effect on isolated guinea pig ileum
11. Effect on cardiovascular and respiratory system in dog (intraduodenal)
12. Charcoal propulsion test in mice (oral)
13. Effect on urinalysis in rats (oral)

Pharmacokinetics

14. Whole body autoradiography of adapalene 0.1% gel.
15. Skin metabolic profile of 0.1% adapalene (topical).

Toxicology

16. Acute toxicity in dogs (oral)
17. Acute toxicity in dogs (cutaneous)
18. Acute toxicity in rats (cutaneous)
19. Primary eye irritation in rabbit
20. Primary skin irritation in rabbit
21. 28-days dermal study in rabbits

Immunotoxicology

22. Antigenicity in guinea pigs
23. Antigenicity in mice
24. Delayed contact hypersensitivity in guinea pigs
25. Phototoxicity and photoallergy in guinea pig

2.6.2 PHARMACOLOGY

Brief summary: Adapalene a synthetic analog of retinoic acid selectively binds to RARβ and –γ nuclear receptors of retinoic acid, however, it does not bind to CRABPII (cellular retinol-binding protein II). In addition to displaying typical retinoid effects (e.g. normalization of the maturation of follicular epithelium), adapalene also exhibits anti-inflammatory properties. Like retinoic acid, adapalene also activates nuclear receptors and inhibits transglutaminase I, an enzyme involved in the terminal differentiation of keratinocytes. The drug also expressed comedolytic activity in Rhino mouse containing a high density of spontaneous "comedones".

Adapalene at oral dose levels of 10, 30, and 100mg/kg did not affect the functioning of central, autonomic, respiratory, cardiovascular, renal or digestive systems in CD-1 mice. At the same dose levels, the same pattern was observed in beagle dogs.

2.6.2.2 Primary pharmacodynamics

The biochemical and pharmacological profiles of the napthoic acid class (e g., adapalene) of agents are similar to retinoids and some other anti-inflammatory drugs. Animal studies have indicated that adapalene is a potent modulator of cellular differentiation, keratinization, and inflammatory processes, all of which represent important features in the pathology of acne vulgaris. Thus, topical adapalene normalized the differentiation of follicular epithelial cells resulting in decreased microcomedone formation. In human keratinocytes, adapalene inhibited the activity of transglutaminase I, a membrane associated enzyme involved in the terminal differentiation of keratinocytes (i.e. formation of stratum corneum). The data of both *in vivo* and *in vitro* studies had revealed that the drug inhibited the chemotactic (directional) and chemokinetic (random) responses of human polymorphonuclear leukocytes. It also inhibited the lipoxidation of arachidonic acid to inflammatory mediators.

2.6.2.3 Secondary pharmacodynamics

Adapalene in 0.1% gel form was efficient in repairing the signs of UVB-induced photodamage (acanthosis, inflammation, elastosis etc.) on the skin of hairless mice.

2.6.2.4 Safety pharmacology

The gavage doses (10, 30, 100mg/kg) of adapalene did not affect the behavior, physical health, spontaneous locomotor activity, hexobarbital sleeping time, pain response, basal tone of ileum, and gastrointestinal motility in CD-1mice. However, between post-dose hours 2-5 at the mid- and high dose levels, drug caused a moderate transient decrease in body temperature. The oral doses at the same levels did not affect the functioning of the cardiovascular, respiratory, and central nervous systems in beagle dogs, and urine volume and electrolyte excretion in Wistar rats.

Abuse liability: Not known

2.6.2.5 **Pharmacodynamic drug interactions:** No studies were conducted

2.6.2 PHARMACOLOGY TABULATED SUMMARY

Receptor binding and gene transcription

Study titles:

1. Determination of retinoids affinity for the retinoic acid nuclear receptors RARα, RARβ and RARγ on hydroxylapatite (HTP) gel (Report # RDS.03.SRE.15989.RO1, study date: 01-21-2002)
2. Determination of retinoids effect on the retinoic acid nuclear receptors RARα, RARβ and RARγ in transactivation assay (RDS.03.SRE.15992.RO1, 01-31-2002).
3. CD271: Determination of retinoids affinity for the cytosolic retinoid acid binding protein CRABPII (RDS.03.SRE.15990.RO1, 01-31-2002).
4. Determination of retinoids effect on nuclear receptors RXR in transactivation (RDS.03.SRE.15991.RO1, 01-31-2002).

Objectives: This group of studies was aimed to compare the affinities and activation effect of adapalene and all-trans retinoic acid on the retinoic acid nuclear receptors RARα, RARβ, RARγ and RXR (retinoic acid orphan receptor), and the cytosolic retinod-binding protein (CRABPII).

Methods: The affinities of retinoic acid, adapalene and the reference compound CD2043 (Tetramethyl-tetrahydro-naphthalen benzoic acid) towards the receptor proteins were determined by comparing with the ligand ^{3}H-CD367 [4-(5, 5, 8, 8-(Tetramethyl-5, 6, 7, 8-tetrahydro-anthracen).

RARβ nuclear protein was extracted from Cos7 cells (epithelial African green monkey kidney cells transformed by SV_{40} virus). The nuclear receptors RARα and RARγ were extracted from SF9 cells (insect cells derived from the pupal ovarian tissue of the fall army worm *Spodpetra frugipedra*). CRABPII was obtained by digesting the transfected (with vector psG5mCRABPII) Cos7 cells.

The radioactive ligand was separated from the free molecule on hydroxylapatite gel. The IC_{50} values for adapalene, retinoic acid and the reference compound were determined. This value correspondence to the concentration of non-radioactive ligand needed to remove half of the specific radiolabeled ligand.

The activation of gene transcription was measured using Cos7 cells transiently transfected by the nuclear receptor RXR. The determinations were made by reading the activation of luciferase luminescence after 18 hours of treatment. The AC_{50} value was determined. This value

corresponds to the concentration of a ligand, which achieves 50% of the maximum activation obtained compared to the reference agonist.

Results: Compared to all-trans retinoic acid, adapalene exhibited poor affinity for RARα, however, it expressed a distinct affinity for β and γ receptors. In contrast, adapalene did not express any affinity to CRABPII. Both compounds activated gene transcription on three nuclear receptors; however, retinoic acid was more effective. None of the compounds indicated any significant gene transcription of RXR.

Cell differentiation

5. Study title: Analysis of the effects of retinoids on the expression of epidermal transglutaminase in cultured normal human keratinocytes (RDS.03.SRE.0213.R01, 03-06-2002).

Objective: In *vitro* study was aimed to investigate the inhibitory effect of adapalene on the epidermal transglutaminase I (Tgase I) in cultured human keratinocytes. TGase I, a membrane-associated enzyme is involved in the terminal differentiation of keratinocytes (i.e. formation of stratum corneum). All-trans retinoic acid is known to inhibit Tgase I in cultured human keratinocytes.

Methods: The keratinocyte cultures prepared from adult human skin were incubated for 4 hours with various concentrations of adapalene, all trans retinoic acid and the retinoid competitor ^{3}H-CD367. The mouse monoclonal antibody B.C1 (ELISA test) was used to determine the level of Tgase I. The IC_{50} value was determined for each of the substrates. This value corresponds to the concentration of a retinoid required to inhibit 50% of the Tgase I signal.

Results: Adapalene inhibited Tgase I with an IC_{50} of 0.6nM. The corresponding value for all-trans retinoic acid was 25nM.

Anti-inflammatory activity

Study titles:

6. Evaluation of the anti-inflammatory activity of CD271 on the arachidonic acid-induced ear edema in BALB/c mouse (RDS.03.SRE.15955, 01-23-2002).
7. Evaluation of the anti-inflammatory activity of adapalene on TPA-induced mouse ear edema (RDS.03.SRE.16182, 07-30-2002).

Methods: The anti-inflammatory action of adapalene was tested on ear edema induced by arachidonic acid (AA) and phorbol-12-myristate-13-acetate (TPA) in 8-9 weeks old female BALBc mice. Indomethacin and

betamethasone valerate were employed as positive controls for AA and TPA assays, respectively. Groups of 6-10 mice received single application of AA/TPA or positive controls in association with AA/TPA, or adapalene in association with AA/TPA, or vehicle alone.

Ear thickness was measured at 0, 1, 2, and 4 hours post-application in case of AA and at 6 hours in the TPA assay. The activity of each compound was expressed as percentage of inhibition of edema induced by AA or TPA.

Results: Whereas the inhibitory action of adapalene on AA-induced edema was statistically non-significant, it exhibited a dose-dependent anti-inflammatory activity in the TPA assay.

Repair of photodamage

8. Study title: The effects of adapalene and all-trans retinoic acid on UVB-induced photodamaged skin in the hairless mouse (2.CG.03.SRE.8002, 2-15-1993).

Objective: This study was conducted to investigate the regenerating activity of adapalene on the UVB-induced photodamage in hairless mouse. All-trans retinoic acid was used as a positive control.

Methods: Five groups of mice (n=12) were subjected to UVB irradiation 3 times per week for 10 weeks. Animals received a total of 5-6 J/cm^2. After that animals received five daily topical applications of 0.1% adapalene gel, placebo gel, 0.05% all-trans retinoic acid lotion, or vehicle for retinoic acid lotion for 10 weeks. The negative control group was not treated. Pictures of treated and untreated mice were taken prior to study initiation, after irradiation and at the end of treatment.

The skin regenerating activity of two test compounds was measured in terms of 1) increase in thickness, 2) the number and size of repair zone (focal or nonfocal areas of neo-collagen arranged in bands, and 3) a decrease in clinical signs.

Results: The ability of adapalene to repair photodamaged skin was greater than the all-trans retinoic acid.

2.6.4 PHARMACOKINETICS/TOXICOKINETICS

2.6.4.1 Brief summary

Irrespective of the formulation and drug concentration, the average topical absorption of adapalene in most species did not exceed 5 percent. The systemic absorption in rabbits was higher. The drug and its metabolites were

mainly distributed in tissues and organs involved in metabolism and excretion. Adapalene did not exhibit any affinity for lipid-rich or melanin-containing tissues or organs. In the single-dose study, the drug related radioactivity in amounts of 20-30ppm (of the applied dose) was retained in the adrenal glands (mainly in the cortex), and in spleen of rats and male rabbits, and thymus and ovaries of rats. However, no such accumulation was observed upon repeated drug administration. Adapalene is extensively metabolized in animals and humans, however, its metabolic pathways and metabolites have not been characterized.

In rats, the placenta acted as a partial barrier to drug and its metabolites during organaogenesis and thereafter. Adapalene is secreted in the milk.

2.6.4.2 Methods of Analysis

[see under individual study reviews]

2.6.4.3 Absorption

Following a single topical dose of 0.6mg/kg [^{14}C]-adapalene, the detectable amounts of the parent drug were found in the plasma of mouse, rat, rabbit, and dog (level of detection 0.15ng/mL). The single dose topical mass balance studies with 0.6mg/kg [^{14}C]-adapalene (from 0.1% solution) under occlusion indicated absorption of 2.5 to 8% in hairless nu-ICO rats. However, the repeated daily applications of the same dose increased the absorption up to 12%. Among the tested species (mice, rats, rabbits, and dogs), the absorption in rabbits (up to 14%) was greater. The bioavailability via the dermal route was also greater (4%) in rabbits than rats (2%).

In a 26-week gavage study in rats, with a T_{max} of 2-3 hours, approximate bioavailabilities at dose levels of 0.15, 1.5, and 15mg/kg/day, were 75%, 17%, and 3%, respectively. In a rat teratology study with 0.1% aqueous topical gel (0.6, 2.0, and 6.0mg adapalene/kg), the bioavailability on day 10 was about 10%. In a repeated dose study (25mg adapalene/kg/day) in rabbit, the absolute bioavailability was about 1%. In a single dose oral study in beagle dogs, the systemic bioavailability of 4-5% was achieved.

Following the oral radioactive doses (0.1 and 1.0mg/kg) of adapalene to pregnant rats, the peak drug levels in the mother and fetus were achieved at 3 and 4 hours, respectively. The amount of radioactivity in the plasma of fetus at 1 hour accounted for 4% of the maternal plasma radioactivity. The $t_{1/2}$ in the mother was about 14 hours at both dose levels, while in fetus, the values were 29 and 40 hours for the low and high doses, respectively.

No drug was detected in the plasma of women treated topically with 2g of 0.1% adapalene gel per day for 3 months. The absorption studies with

human skin preparations or keratinocytes cultures revealed a slightly higher absorption (never exceeding 10%) than in majority of the intact animals.

2.6.4.4 Distribution

In single and multiple-dose [^{14}C]-adapalene plasma kinetic studies in Sprague-Dawley (S-D) rats, irrespective of the dose (0.12-0.5mg/kg), vehicle (PEG-400, CMC, or gel) or route (intravenous, oral, topical) more radioactivity was found in the plasma of females. In a 21-day topical rat study, a steady-state plasma drug level was achieved in males (0.92ng/mL) and females (1.21ng/mL) at days 8 and 13, respectively.

Following a single topical application of 0.3% adapalene solution in male S-D rats, at 24 hours post-dose, approximately 7% of the applied dose was found in the skin. Out of it 3% was present in the *stratum corneum*. It was indicated that the radioactivity diffused from the *stratum corneum* to dermis and hypodermis and also to a very little extent (<0.1% of the dose applied) to the subcutaneous tissue. After 7 days, the amount in the skin was reduced to 0.1%. The apparent $T_{1/2}$ of elimination ranged between 3 to 4 days. In a similar study in rabbits, the corresponding amounts on days 1 and 7 were 3.5 and 1%, respectively. The maximum amount of radioactivity was found in the *stratum corneum*, which assumably acts as a reservoir for drug release.

In a 28-day topical rat study (0.1mg/site) with 0.1% adapalene solution, after the last application, approximately 2% of the total dose was present in the skin. The amount of radioactivity found in the tissues accounted for 0.06-0.08% of the administered dose. The elimination half-lives in the adrenals, ovaries, spleen, and uterus were much longer than plasma.

Seven days after the intravenous dose in male rats, rabbits and dogs, adrenals, liver, bile and spleen contained more radioactivity than the plasma. Each species exhibited a characteristic pattern of distribution, whereas in rat the highest amount of radioactivity was found in the adrenals, in dogs the liver and fat contained the highest amounts. The amount of radioactivity decreased rapidly in all tissues and organs, except for adrenal glands, thymus, and ovaries of rats and rabbits. The radioactivity in the adrenals was mostly retained in the cortex. However, the amount retained was very small, typically 20-30ppm of the applied dose at 72 hours post-dose.

The tissue distribution data following a dermal whole body autoradiography study revealed accumulation of drug-related radioactivity mainly in organs and tissues involved in the metabolism and excretion.

In rats, the placenta formed a relatively good barrier for drug and metabolite-related radioactivity after single and repeated dosing during organogenesis and upon single dosing during late pregnancy.

In vitro study with human blood, 26% of the ^{3}H-adapalene was bound to erythrocytes and the total binding in blood was more than 99%, mostly to lipoproteins and albumin.

2.6.4.5 Metabolism

Adapalene is extensively metabolized by human hepatocytes. The metabolism in dogs is very similar to men. The data from several studies had indicated that metabolism probably affects only the methoxybenzene moiety. However, out of 7 fecal metabolites, only one has been identified. Adapalene did not interact with cytochrome P450, nor it exhibited any potential for enzyme induction.

2.6.4.6 Excretion

After the intravenous administration of [^{14}C]-adapalene, glucuronides, a sulfo-conjugate, and the parent drug represented 63.2, 17.1, and 19.1% of the metabolic pool in the bile of rats. In an enterohepatic circulation study, 3-6 hours after the intravenous dose of radioactive drug into the rat duodenum, 75% of the metabolic pool was in the free form, and 24.1% had undergone glucuronidation; sulfonation was almost negligible. After reabsorption, the compounds were once again eliminated via the fecal route, indicating the existence of a considerable enterohepatic circulation of adapalene and its metabolites. The trace amounts of radioactivity found in the urine of mouse, rat, and dog did not permit detailed analysis. In rabbit, about 5% of the radioactivity following the intravenous dose was found to consist largely of polar compounds (55.3%).

In four volunteers, the total amount of radioactivity found in the feces following a topical application of 0.1% adapalene solution amounted to 0.02-0.06% of the applied dose. No significant amount of radioactivity was found in the urine.

2.6.4.7 Pharmacokinetic drug interactions: Not investigated

2.6.4.8 Other Pharmacokinetic Studies: N/A

2.6.4.9 Discussion and Conclusions:

Irrespective of the route of administration, female rats absorbed more adpalene. The bioavailability was also greater in females, however, it was much less via the dermal route. At the same concentration level, the dermal

absorption in rabbits was almost 3 times more than in rats, however, the bioavailability was only 1.7 times greater. No drug was detected in the plasma of women treated with 2 grams of 0.1% adapalene gel per day for 3 months.

In animals as well as humans, the amount of drug absorbed via the dermal route caused no systemic toxicity. The local lesions related to retinoid like action of adapalene were not severe in nature.

2.6.4.10 Tables and figures to include comparative TK summary:

Repeated topical dose (6mg/kg/day) pharmacokinetics in animals

	Rat	Rabbit	Dog
Cmax (ng/mL)	14	48	20
$AUC_{0\text{-}24}$ (ng.h/mL)	204	1036	--
F (%)	2	4	--

2.6.5 PHARMACOKINETICS TABULATED SUMMARY

Pivotal studies: The dose selection in these studies was based on the previously conducted teratology studies.

1. Pharmacokinetics study of Adapalene Gel 0.3% in female Sprague-Dawley rat after topical application during 10 days (RDS.03.SRE.31008, 12-05-2003).

Materials and Methods:

The pre-shaved dorsal sites (12 cm^2) on the back of 6 weeks old OFA S-D female rats received daily topical applications of 6mg adapalene/kg (2mL/kg of 0.3% adapalene gel) for 10 days. Blood samples for the determination of plasma drug levels were drawn pre-dose, and at 1, 3, 6, 10 and 24 hours post-dose on day 10.

Results/Conclusions: Cmax (14ng/mL) achieved at 1 hour indicated a rapid drug absorption. The plasma drug concentration reached a plateau by 6 hours post-dose. The systemic exposure ($AUC_{0\text{-}24\,h}$) was 204 h.ng/mL.

2. [^{14}C]-Adapalene: Plasma kinetic studies in the rat following single intravenous, oral, topical, and repeated topical administration (1.CG.03.SRE12104, 11-29-2000).

Materials and Methods:

Animals: Sprague Dawley CD rats
Protocols: Table 1

The pre-shaved application sites were semi-occluded for 6 hours post-dose and animals wore collars through out the study period except during dosing. In-groups 1 and 2, blood samples were drawn from 3 rats/sex. In-group 3, excreta samples were collected daily and at 24, 48, and 96 hours after the last application. This study was aimed to monitor absorption, excretion, and the total recovery of radioactivity. The steady state kinetics was investigated in-group 4. The plasma drug levels in-group 5 were used to determine the pharmacokinetic parameters after completion of 3 weeks of dermal applications.

Table 1.

Group	no	Dose/route/frequency	Dose level	Blood sampling
1.	48/sex	Intravenous/single	0.5mg/kg of [^{14}C]-adapalene	At 16 points between 0.083-72 hours
2.	48/sex	Oral/single	Same as in #1	Terminal at 16 points (0.25-72 hrs)
3.	3/sex	Topical/21daily	120 mg/day/rat of 0.3% radioactive gel (~0.36mg/kg adapalene)	Terminal at 3 points
4.	6/sex	Topical/21daily	120mg/day/rat of 0.3% unlabelled gel (~0.36mg/kg)	Prior to dose on days 2, 4, 8, 13, 16, and 21
5.	9/sex	Topical/21 daily	Same as above	At nine time points after the last dose

Results: Irrespective of the route of administration, females had a greater ($p<0.003$) exposure (AUC values) to adapalene than males (Table 2). The bioavailability was also greater in females. However, it was 5% or less via the topical route. In the 21-day topical study, the plasma drug levels reached steady-state by day 8 in males (3-5ng/mL), and by day 13 in females (6-9ng/mL). The terminal half-lives in both the sexes was similar.

In the second 21-day topical study, a major portion of the administered radioactivity remained unabsorbed (Table 3). A less than 100% recovery of radioactivity was attributed to the protein binding nature of adapalene, also observed in other mass balance studies with this drug.

Table 2. Comparative pharmacokinetics

Pharmacokinetic Parameter	Intravenous		Oral		Topical	
	Males	Females	Males	Females	Males	Females
Cmax (ng.mL)	706	559	15.7	15.8	3.64	7.38
Tmax (hours)	0.08	0.08	3.0	4.0	6.0	8.0
AUC_{0-24} (ng.h/mL)	858	1002	-	-	56.8	114.6
AUC_{0-96} (ng.h/mL)	-	-	-	-	174.6	327.2
AUC∞ (ng.h/mL)	-	-	82.8	104.4	-	-
$T_{1/2}$ (hours)	-	-	2.5	2.7	-	-
F (%)	-	-	8.9	9.7	3.7	4.9

Table 3. Total recovery of radioactivity (% of applied dose) after 21 daily topical applications (Group 3).

	Males	Females
Urine, feces, cage wash	5.12 (2.52-10.08)	10.60 (7.61-13.71)
Carcass & GI tract	0.13 (0.09-0.20)	0.17 (0.13-0.22)
Untreated skin	0.33 (0.16-0.60)	0.31 (0.22-0.38)
Treated skin	0.56 (0.29-1.04)	0.43 (0.29-0.66)
Skin swabs, dressing	79.32 (72.07-84.07)	72.53 (64.84-78.24)
Total recovery	85.46 (82.84-87.36)	84.04 (79.35-87.02)

3. Pharmacokinetic evaluation of 0.3% Adapalene gel formulation following dermal administration to the female rabbits for 14 days (RDS.03.SRE.31009, May 2003).

Materials and Methods: Six NZW rabbits of each sex (~7 weeks old, 2.5-4kg bw) received daily topical applications of 0.3% adapalene gel (2mL/kg=6mg adapalene/kg) on the pre-shaved dorsal sites ($10cm^2$) for 14 consecutive days. Applications on days 1-6 were made on site #1, on days 7-12 on site #2 and on days 13 and 14 on site #1. The exchange of sites became essential because of the development of some dermal lesions after the first 6 applications. During 6 hours of exposure period, animals wore collars. Blood samples for the determination of plasma drug levels were drawn pre-dose, and on day 14 at various time points between 0.5 to 24 post-dose hours.

Results: Some dermal reactions (erythema, erosion, desquamation) on the application sites were observed in all rabbits after the second dose. Some animals also exhibited pain on touch. According to the sponsor, such reactions are typical of this kind of retinoid compounds.

The pre-dose level of plasma adpalene on day 14 was 36ng/mL. Cmax of 48ng/mL was achieved at one hour. Thereafter, a plateau developed, and the level reduced very slowly. The level at $AUC_{0-24\,h}$ was 1036 h.ng/mL.

4. [^{14}C]-Adapalene: Absorption, distribution and excretion studies in dog following single intravenous, oral and topical administration (1.CG.-03.SRE.12109, June 2001).

Materials and Methods: Approximately 15 months old beagle dogs (4/sex) received a single dose of [^{14}C]-adapalene via the intravenous, oral, and topical routes. Same animals were used for each experiment after 4 weeks of wash-out period between dosing.

In the intravenous study (0.5mg/kg), blood samples for the determination of plasma drug levels and radioactivity were collected pre-dose and at 18 post-dose time points. The excreta samples were collected daily for up to 13 days. In the oral study (0.5 mg/kg), blood samples were also collected at the same time points, however, excreta samples were collected for up to 8 days. The topical application (5mg/dog= 2-3mg adapalene/kg) was made on the semi-occluded pre-shaved site. Blood and excreta samples were collected as in the oral study. After sacrifice on day 8, treated and untreated skin sites were excised.

Results: Following the topical application, the plasma drug concentration was below the detection limits, indicating negligible to no dermal absorption. Most of the radioactivity (87-90%) in this case was found in the swabs and dressings used on the application site. Less than 0.3% of the administered dose was excreted, and approximately 2% remained on the application site.

After the intravenous dose, most of the radioactivity (80%) excreted in the feces, and less than 1% was found in the urine. Approximately 0.1-0.2% of the radioactivity was still excreted in the urine at 9-12 days post-dose.

Following the oral dose, 81-83% and 0.1% of the radioactivity was excreted in the feces and urine, respectively.

In general, after the oral dose, the values of the evaluated pharmacokinetic parameters were greater in females (Table 1). At least 50% of the radioactivity in the plasma was due to metabolites.

Table 1. Pharmacokinetic data after a single intravenous and oral dose to dogs.

Pharmacokinetic Parameter	Intravenous		Oral	
	Males	Females	Males	Females
Cmax (ng/mL)	2230	2350	35.7	42.1
Tmax (h)	0.08	0.08	3.0	4.0
AUC_{0-168} (ng.h/mL)	5489	4644	562	760

AUC∞ (ng.h/mL)	5834	4812	714	1372
K_{el} (h-1)	0.03	0.02	-	-
$t_{1/2}$ (h)	25.5	27.6	-	-
CL (mL/min/kg)	1.37	1.93	-	-
V_z (L/kg)	3.07	5.58	-	-
V_{ss} (L/kg)	0.54	1.08	-	-
F (%)	-	-	6.6	9.4

5. Quantitative whole body autoradiography (QWBA): Tissue distribution studies in the pregnant OFA Sprague Dawley rat following single intravenous administration of [^{14}C]-adapalene at the dose of 0.5mg.kg^{-1} (1.CG.03.SRE.12184, November 1999).

Objective: To assess the potential placental transport of drug and its metabolites by comparing the radioactivity (AUC) in maternal and fetal tissues.

Materials and Methods: The tissue distribution of radioactivity was determined following a single intravenous dose of 0.5mg/kg of [^{14}C]-adapalene (radioactive purity [redacted] %) on gestation days 11and 18 (mid-organogenesis and postorganogenesis periods, respectively) using yolksac and choriollantoic placentas. Prior to drug administration, females were starved for 15 hours. The distribution of metabolic pool was determined by whole body autoradiography using post dose time points between 1-72 and 1-24 on day 11 and day 18, respectively. Three rats were used at each time point. The radioactivity was determined in the following target organs:

Day 11: mammary glands, maternal plasma, decidua, amniotic fluid, uterus, placenta, and embryo.

Day 18: maternal plasma, mammary glands, uterus, amniotic fluid, placenta, fetal brain, fetal gut content, brain, liver, and fetus.

The elimination of radioactivity was determined on day 13 in a satellite group (n=3).

Results: The pharmacokinetic profile was almost similar on gestation days 11 and 18 (Tables 1 and 2). The highest amount of radioactivity was found at one hour post-dose in all the tissues selected for assessment. The mammary glands did not register on the imaging plates because the labeling was below the background level.

The plasma drug concentration after Tmax (1 hour) declined with time to reach 3 (72 hours) and 10 ng eq.g^{-1} (24 hours) on day 11 and day 18, respectively (data not shown). On day 11, the drug concentrations in the yolksac placenta (vascular part) and plasma were approximately similar

with a ratio of 1.1 at one hour post-dose. However, on day 18, the vascular part of placenta contained much more radioactivity with tissue plasma ratio of 1.4 (1 hour) to 3.7 (24 hours). The AUC_{0-24} was approximately four folds greater than the plasma. This could be attributed to the placenta type. In rats, the chorioallantoic placenta starts to develop on day 12 and become progressively functional, contributing to a gradual increase in transfer of xenobiotics. On day 18, the amniotic fluid contained 18 times (AUC_{-inf}) lower radioactivity than day 11.

The drug exposure (Cmax) in fetus on day 18 was 4 folds greater than embryo on day 11. The greater exposure in fetus could be linked to the formation of new compartments thus increasing the total body burden.

The fetal liver exhibited high labeling due to the presence of parent drug, its metabolites and excretory products. However, the hepatic concentration-time curve (333, 307, 270, and 65 ng eq. g^{-1} at 1, 4, 8, and 24 hours) appeared to be a linear decrease, indicating a saturation of excretory elimination process.

With time, the radioactivity decreased in all the tissues except for the GI content where the peak was achieved at 24 hours post-dose.

Approximately 92 and 0.5% of the administered dose was eliminated in the feces and urine, respectively. At 72 hours, the total excretion of radioactivity (urine, feces, and carcass) was 95 percent.

Table 1. Pharmacokinetics on gestation day 11

Parameter	Plasma	Amniotic fluid	Embryo	Placenta	Uterus	Decidua
Cmax ($ng.eq.g^{-1}$)	486	57	57	553	387	832
Tmax (hour)	1	1	1	1	1	1
$AUC_{0-72\,h}$($ng.eq.h.g^{-1}$)	3003	1033	1033	3498	4712	9176
AUC_{0-inf}($ng.eq.h.g^{-1}$)	3071	1499	1499	3738	5010	9654
Kel(h^{-1})	0.04	0.01	0.01	0.03	0.03	0.04
$AUMC_{0-72h}$($ng.eq.h^2.g^{-1}$	6490	64978	64978	25618	31622	47617
$AUMC_{0-inf}$($ng.eq.h^2.g^{-1}$)	17	47	47	24	24	19
$T_{1/2}$(h)	12	60	60	18	19	24

Table 2. Pharmacokinetics on gestation day 18

Parameter (units as in table 1)	Plasma	Amniotic Fluid	Placenta	Uterus	Whole Fetus	Fetal brain	Fetal liver	Fetal Memb
Cmax	586	8.02	823	352	211	161	333	461
Tmax	1	4	1	4	8	4	1	8
AUC_{0-24h}	2806	77	10407	3515	3470	1154	4574	7658
AUC_{0-inf}	2868	84	13508	3733	4413	1227	5575	11164
Kel	0.14	0.11	0.06	0.12	0.07	0.12	0.08	0.05
$AUMC_{0-24}$	11353	599	92428	25397	34113	8048	42032	78865
$AUMC_{0-inf}$	13293	832	216819	33819	71087	10386	71899	237190
$T_{1/2}$	5	6	11	6	11	6	9	15
MRT(h)	5	10	16	9	16	8	13	21

6. CD 271 (Adapalene): *In vitro* metabolism studies (rat) and structural identification of two metabolites (rat and human) (RDS.03.SRE. 4518, August-September 1994).

7. *In vitro* metabolism of CD271 by male rat hepatic microsomes (CF/JF/90-244, January-February 1990).

Objective: These studies were aimed to investigate the metabolic profile of adapalene using human hepatocytes and rat liver microsomes. The rat liver slices were used to isolate various metabolites for chemical characterization.

Materials and Methods: Seven batches of human hepatocyte cultures were incubated with 5µM of [^{14}C]-adapalene for up to 7 days. The aliquots were used to determine the total radioactvity by liquid scintillation counting in each batch. The incubated medium was treated with [ ] [ ] to deconjugate any metabolites present in the conjugated form. The various metabolites were separated by HPLC.

The rat liver slices incubated with [^{14}C]- adapalene did not provide sufficient amount of any metabolite needed for characterization.

The liver microsomal pellets prepared from male Sprague-Dawley rats were incubated with [^{14}C]-adapalene dissolved in DMSO. All reactions were started with the addition of a NADPH generating system. A portion of each incubate was used for the qualitative analysis of metabolites by thin layer chromatography, rest was used to separate metabolites by high-pressure liquid chromatography (HPLC).

Results/Conclusion: The incubated human hepatocyte media contained conjugated metabolites. The media was resolved by HPLC to a total of 6 metabolite fractions (A-F). The more polar and more hydrophobic

compounds were present in fractions A-C and D-G, respectively. The A and C fractions primarily contained glucuronide material. The B fraction was similar to a previously isolated metabolite M2, a dihydroxylated moeity with hydroxylation occurring on the adamantyl group. The fraction D was not characterized. The fraction F contained a metabolite with a polarity very similar to the parent drug. The G fraction contained the parent drug.

Three major metabolites produced by the rat liver microsomes were designated as M7, M6, and M4.

These studies failed to completely characterize any of the metabolites.

8. The effects on selected hepatic enzyme activities and related parameters in the rat following oral gavage administration for 14 days (1.CG.03.SRE.12108, September 2001).

Objective: The potential inducing effect(s) of adapalene on selected hepatic microsomal enzymes were investigated.

Materials and Methods:

Animals: Approximately 7 weeks old male (236-250g) and female (170-182g) Sprague-Dawley rats.
Vehicle: 0.5% aqueous carboxymethylcellulose+0.1% Tween 80
Test groups (n=6rats/sex/group):

1. Vehicle control (dose volume 10mL/kg)
2. Phenobarbital (+ve control) 75mg/kg/day
3. Adapalene 0.15, 1.5, 15, or 50mg/kg/day

Animals received daily gavage doses of respective test substance for 14 consecutive days. At the end of treatment, microsomal subcellular fraction was prepared from each liver by differential centrifugation.

Parameters determined:

1. Concentration of cytochrome P450
2. Concentration of protein
3. Activities of the following P450 enzymes: CYP1A, CYP2B and 3A (testosterone metabolizing enzymes), CYP2E and 4A (lauric acid hydroxylase), and UDP-glucuronyltransferase.

Results: Phenobarbital produced a significant increase in the activities of most of the CYP450 and glucuronyltransferase enzymes thus validating the assay. Adapalene at the highest dose level significantly increased the activities of lauric acid 11- and 12-hydroxylases (CYP2E1 and CYP4A) in males. No biological significance was linked to this change.

9. [^{14}C]-Adapalene: Excretion studies in the rat following single intravenous, oral, topical and repeated topical administration (1.CG.03.SRE.12105, November 2000).

Materials and Methods:

Animals: Male (224g) and female (194g) Sprague-Dawley rats
Test substance: [^{14}C]-adapalene
Test groups:

1. Single intravenous (0.5mg/kg) dose in PEG 400/ethanol/0.9% NaCl (7:1:2 w/w). Daily excreta samples were collected for 7 days. Blood samples were collected at 6 hours post-dose.
2. Single oral dose (0.5mg/kg) in 0.5% aqueous CMC containing 0.1% Tween 80. Excreta and blood samples were collected as in group 1.
3. Single topical (0.12mg/rat) application of 0.1% Adapalene gel. Excreta and blood samples were collected as in-group 1. The application site (12 cm^2) was occluded for 6 hours.
4. Daily topical dose of 0.12mg/application site for 21 consecutive days. The application sites were occluded for 6 hours.

Results: Following the intravenous injection, approximately 88% of the administered dose was excreted in the feces within 72 hours, however, the radioactivity was still found in the feces at the end of study. Only 0.1 to 0.2% of the radioactivity was found in the urine. The carcasses contained approximately 3% of the radioactivity. The overall recovery of radioactivity was 91-92 percent.

Almost 100% of the radioactivity was excreted in the feces after the single oral dose. Most of it (~80%) excreted within the first 24 hours was due to parent drug. The carcasses contained less than 0.2% of the administered dose on day 7.

After the single topical dose, approximately 60% of the radioactivity was found in the swabs and dressings etc. The amount in the plasma was below the detection limits. Less than 0.5% of the radioactivity was found in the excreta at 48 hours. After this time point, the radioactivity found in the feces was considered to be due to oral ingestion of residual dose from the application sites. The overall recovery of radioactivity was around 91 percent.

In the multiple topical application study, at 6 hours post-dose, the plasma in males and females contained 2.2 and 4.9ng drug-related radioactivity/g, respectively. On day 7, the feces contained 32-51% of the administered dose. Most of it was due to ingestion from the application sites.

10. [^{14}C]-Adapalene: Milk secretion studies in the rat following single intravenous and oral (gavage) administration (1.CG.03.SRE.12107, May 2000).

Materials and Methods: On day 14 of parturition, a group (n=14) of lactating CD rats received a single intravenous dose of [^{14}C]-adapalene (0.5mg/kg) in PEG 400+ethanol+saline mixture. Milk samples were drawn from 3rats/time point at 0.5, 1, 3, 6, and 24 hours post-dose. Thereafter, the blood samples were collected at the same time points.

A second group of 18 lactating rats received a single oral dose (15mg/kg) of the radioactive drug in aqueous CMC suspension on day 14 of parturition. Milk samples were drawn at 1, 3, 6, 24, 48, and 72 hours. The terminal blood samples were also collected.

Results: In the intravenous study, the peak concentration (543ng eq./g) of radioactivity in the plasma was achieved at the very first time point (1 hour). The drug in the milk was secreted between 0.5 and 24 hours with the peak concentration (312ng eq./g) reaching at 3 hours. Up to 6 hours, relatively high amounts of radioactivity persisted in both the fluids, by 24 hours it was declined to 2% of the peak concentration.

In the oral study, the peak concentrations in the plasma (265ng eq./g) and milk (266ng eq./g) were achieved at 3 and 6 hours post-dose, respectively. At 48 hours, the plasma drug level was reduced to 1% of the peak concentration. The decline in milk radioactivity occurred more rapidly, at 24 hours the amount was approximately 2% of the peak concentration, and by 48 hours the level was below the detection limits.

2.6.6 TOXICOLOGY

2.6.6.1 Overall toxicology summary

General toxicology: The acute oral LD_{50} for adapalene in both sexes of S-D rats and CD-1 mice was greater than 5,000mg/kg. The acute LD_{50} of adapalene applied as 0.3% solution was greater than 10mL/kg (30mg/kg) in [ ] male and female mice. In an acute dermal study in rats, 2grams/kg of 0.3% adapalene gel did not produce any systemic and or local toxicity.

In two separate studies where [ ] mice received gavage (110, 300, and 500mg /kg) and intraperitoneal doses (30, 60, and 80mg/kg) of adapalene for two weeks (10 doses), hypervitaminosis A syndrome characterized by loss of hair, body weight, spontaneous long bone fractures and skeletal resorption were observed.

The topical applications of 0.3% adapalene gel in rats at the maximum feasible dose of 2 mL/kg (6mg adapalene/kg/day) for 4-26 weeks did not cause any systemic toxicity. The dose-related scabs and acanthosis disappeared during 8 weeks of recovery period.

In a 26-week gavage study (0.15, 1.5, and 15.0mg adapalene/kg/day) in CD rats, the drug related toxicity was restricted to a slight decrease in the number of erythrocytes in high dose females and an increase in the plasma alkaline phosphatase at the highest dose level in both sexes. Irrespective of the high plasma drug levels after the last dose (7.6, 50.2, and 113.4 in males and 9.4, 46.0, 148.6ng/mL in females), no biological effects of any significance were observed.

Dogs treated orally (1mg/kg/day) and topically (6mg/kg/day) for 26 weeks, did not exhibit any bone-related systemic toxicity, however, epidermal hyperplasia and superficial dermal inflammation on the application sites were observed in all the topically treated animals. In another 26-week oral (1-20mg/kg/day) dog study, the changes in bones due to increased osteoclastic activity and resorption resembled hypervitaminosis A syndrome. It was inferred that 1mg oral adapalene/kg/day is close to the threshold dose that causes cranial bone changes in dogs.

Genetic toxicology: Adapalene was evaluated as non-mutagenic in gene mutation assays (Ames and mouse lymphoma tests with/without S-9 fraction), and non-clastogenic *in vitro* (Chinese hamster ovary cells with/without S-9 fraction) and *in vivo* (mouse micronucleus assay at 6,000mg/kg) tests. The dose selection in all studies was based on the dose range-finding studies, and the assays were simultaneously validated with the use of positive controls.

Carcinogenicity: One combined oncogenicity/chronic toxicity dietary study in CD rats and one dermal oncogenicity study in CD-1 mice were conducted.

In the topical study, mice received one daily application of 0.03, 0.1, and 0.3% aqueous gel (equivalent to 0.06, 2.0, and 6mg adapalene/kg/day) for 19 weeks. However, because of the severity of dermal lesions, the frequency of application was reduced to five times a week, and for the same reason was further reduced to three times per week from week 63. A number of animals were sacrificed on humane ground due to severe local reactions. Compared to the vehicle control, the low survival rates in 0.1% males and mice of both sexes receiving 0.3% gel, indicated a positive dose-related trend.

Gross pathological examination revealed thickening of the skin. The histopathologic examination indicated acanthosis, hyperkeratosis, scabs, ulcers, diffused subcutaneous inflammation, collagen deposition, atrophy of glandular and follicular structures, and increased superficial follicles in the drug treated skin. Most of these incidences were statistically significant and dose related. No drug related neoplastic changes were observed.

In the rat dietary study, animals received daily doses of 0.15, 0.5, or 1.5mg adapalene/kg/day for 104 weeks. Significant drug related non-neoplastic changes observed at 78 week interim sacrifice (chronic toxicity phase) included adrenal medullary hyperplasia in both sexes, and centrilobular hepatocytic vacuolation and extramedullary hemopoiesis in the high-dose females. The high incidences of periacinar hepatocytic fatty vacuolation, chronic inflammation of stomach, tubular mineralization of testes, and transitional cell hyperplasia of urinary bladder were observed in the high-dose males. High dose females also exhibited higher incidence of uterine dilation.

The absolute and relative weights of adrenals were significantly increased. The high-dose males exhibited a significant ($p<0.05$) incidence of benign pheochromocytoma of the adrenals. The combined number of benign and malignant pheochromocytoma, and pancreatic islet cell tumors in drug-treated males indicated a higher incidence. According to the sponsor, the high incidence of pheochromocytoma is a characterisitic of compounds acting like retinoids. There are many morphological and biochemical differences between the adrenal glands of the rat and man. Furthermore, the incidence of pheochromocytoma in man is very low (0.005 to 0.09%). A high incidence of carcinomas and adenomas of thyroid was observed in the drug treated females.

No photocarcinogenicity study was conducted.

Reproductive toxicology: In an oral reproductive performance and fertility study where F_0 female rats were treated with daily doses of 1.5, 5, or 20mg adapalene/kg for 15 days prior to pairing and throughout the gestation and lactation periods, no effects on reproductive performance and fertility, F_1 litter size, growth, development to weaning, and subsequent reproductive performance of the offspring, were observed.

In dermal teratology studies with adapalene gels (0.03, 0.1, and 0.3%), the number of ribs in rats and rabbits at the highest dose (6mg/kg/day) level were increased. There were slight increases in the incidence of pre-sacral vertebrae (rabbit), asymmetric pelvis (rat) and small additional fissure in the parietal bone (rat), or more varied anomalies of the interparietal bone (rabbit).

In the oral teratogenicity study in rats (5, 25, and 60mg/kg/day), based on significant skeletal and visceral malformations both mid and high doses were established as teratogenic. At the low dose, only minimal skeletal variations (additional ribs) were observed. This dose was considered to be non-teratogenic.

In segment 3 rat study (0.15, 1.5, and 15mg/kg/day), the highest dose of adapalene had no effect on the litter parameters (e. g. development after weaning, mating and fertility) of F_0 and F_1 generations, and on F_2 fetuses. Since adapalene was also excreted in the milk, it is inferred that the pups were exposed both *in utero* and during lactation.

Special toxicology: Adapalene gel did not exhibit any phototoxicity or photoallergenicity in guinea pigs. Adapalene cream did not induce sensitization in guinea pigs.

2.6.6.2 Single-dose toxicity

Study title: 1. Adapalene 0.3% gel: Acute dermal toxicity study in rats (1.CG.03.SRE.12097, February 1998).

Materials and Methods: Ten Sprague-Dawley rats (5/sex, 246-310g) received a single application of 2 grams/kg of 0.3% adapalene gel on a pre-shaved site (50x50 mm). Following 14 days of observation period, all animals sacrificed on day15 were subjected to gross pathology examination.

Results: Throughout the study period, animals did not exhibit any local and systemic toxicity, and the gain in body weight was normal. The necropsy examination did not reveal any gross abnormalities. The acute lethal dermal dose for 0.3% adapalene gel was greater than 2000mg/kg.

Study title: 2. A primary skin irritation study of CD271G in the rabbit (BOZO/I-1073, April 1998).

Materials and Methods: Fourteen weeks old female (4/group, 2-3kg) Japanese white rabbits received applications of 0.5mL of 0.0, 0.03, 0.1, and 0.3% of adapalene gels on the pre-shaved occluded dorsal sites (2.5cm^2). Two intact and two abraded sites on each rabbit were treated. Observations for skin irritation were made at 24, 48, and 72 hours post-application. Throughout the study period animals were also examined for any clinical signs of toxicity.

Results/Conclusions: No signs of toxicity or change in body weights were observed. A primary skin irritation index of zero at all drug levels indicated that adapalene gel at 0.03-0.3% concentrations was a non-irritant.

2.6.6.3 Repeat-dose toxicity

Study titles: 1. Adapalene gel: Toxicity study by cutaneous administration to CD rats for 4 weeks.
2. Adapalene gel: Toxicity study by administration to CD rats for 26 weeks followed by an 8-week recovery period.

Key study findings: The topical applications of adapalene up to 6mg/kg/day for 4-26 weeks did not cause any systemic toxicity. The scab-formation and acanthosis on the application sites were drug and dose related. All dermal lesions disappeared during 8 weeks of recovery period.

Study nos.: 1.CG.03.SRE.12098, and 1.CG.03.SRE. 12099
Volume #, and page #: 20, 008, and 23, 1
Conducting laboratory and location: [redacted]

Dates of study initiation: April 1998, and October 1998
GLP compliance: Yes
QA reports: yes
Drug, lot #, and % purity: B039-B041/[redacted]%; BO53, BO54, and BO55

Methods

Doses: 0.0 (vehicle control)), 0.6 (0.03% gel), 2.0 (0.1%gel), and 6.0mg (0.3%gel)/kg/day. In study#2, two additional groups (control and 0.3% groups) containing 10rats/sex were used for 8 weeks of recovery phase.
Species/strain: CD rats
Number/sex/group or time point (main study): 10/sex/dose group
Study#2: 20rats/sex/group
Route, formulation, volume, and infusion rate: Topical/0.01, 0.1 and 0.3% gels, 2mL/kg.

Note: According to the sponsor 0.3% is the maximum possible concentration of adapalene in the gel formulation, and 2mL/kg is the highest feasible applicable volume for a repeated dose dermal study.

Satellite groups used for toxicokinetics or recovery: In study one, 3 rats/sex/time point for pharmacokinetics, In study two, 12 rats/sex/group for recovery
Age: 5-7 weeks
Weight: 140-219g
Sampling times: In study one, blood samples were collected on days 1, 7, and 28 for blood. In study #2, blood samples were collected from 10 rats/sex/group in weeks 4 and 26 (main study) and in week 8 of recovery period.

Unique study design or methodology (if any): None

Observations and times: (the parameters are described under each endpoint under results.

Results

Mortality/Clinical signs: In the first study, animals examined at regular intervals did not exhibit any signs of systemic toxicity. Scabs not related to dose were observed on the application sites in a few animals of all groups. No drug-related deaths were recorded.

In the second study, seven rats (5 males, 2 females) of different groups including control were sacrificed on humane grounds at different time points. A few of these animals had collar injuries; other died during blood sampling. One high-dose female was killed in week 25 due to loss of use of hind limbs. Histopathological examination of this rat did not reveal any drug-related cause for death. Two control males were sacrificed during week 8 of recovery period.

In the second study, a dose related scab formation on the application sites was observed in most animals. In addition, erythema in the mid-dose males and flaking of skin in both the sexes at all dose levels were observed. The dermal reactions disappeared during week 3 of the recovery period.

Body weights/Food consumption: In both studies, the weekly determinations did not reveal any treatment-related changes.

Ophthalmoscopy: The examination during week 4 (study#1) and week 25 (study#2) of treatment did not indicate any changes in eye morphology.

EKG: Not conducted

Hematology: In the first study, except for low lymphocyte counts in both sexes at low- and mid-dose levels and in high-dose males, no other drug or dose-related changes in any other hematological parameters were recorded.

In the long-term study, a few statistically significant drug-related changes mostly restricted to mid- and high-dose females were observed. However, there was no dose-related trend. For instance, at week 4 whereas the total leukocyte and lymphocyte counts at the mid- and high-dose levels were reduced by 23 and 26 percent, respectively, they were increased by 23 and 24 percent at week 26. In the high-dose males, an increase in the activated partial thromboplastin time was observed at weeks 4 and 26 (14 and 9%, respectively at $p<0.01$), it increased (24%) at the end of the recovery period.

Clinical chemistry: In females of 4-week dermal study, significant changes in the plasma alkaline phosphatase (ALP), and the ALP isozymes specific to liver (LALP) and intestine (IALP) were observed (Table 1). The changes in ALP and LALP were dose related. The increase in ALP was attributed to a large increase in LALP. No statistically significant changes in males were recorded. A few other significant but randomly distributed changes in the clinical chemistry parameters in both the sexes were not considered biologically significant.

In the long-term study, at week 4 the activities of liver (LALP) and intestinal (IALP) isoenzymes in both the sexes were significantly increased and decreased, respectively (Table 2). This pattern was similar to that was observed in the 4-week study. At week 26, whereas LALP activity increased many folds, the IALP activity was similar to control. In addition, a significant decrease in the plasma cholesterol levels, and increases in triglycerides were observed at both time points.

All the changes recorded at week 26 in the high-dose animals were not observed at week 8 of the recovery period.

Table 1. Summary of clinical chemistry findings (% change from control)
Study # 12098

	Week 4					
Parameter	Males			Females		
	Low	Mid	High	Low	Mid	High
ALP	-15	-15	-12	+4	+30**	+37***
LALP	NC	-7	+2	+61***	+94***	+102***
IALP	NC	NC	NC	-33**	-33*	-33*

Table 2. Summary of clinical chemistry findings ((%change from control)
Study#12099

			Week4			
ALP	NC	NC	+20*	+4	+17	+34
LALP	+19	+34**	+41***	+24	+37**	+81***
IALP	-50***	NC	-50**	0***	-50*	-50**
CHOL	-9	-18*	-33**	-4	-9	-12
TRG	-3	+23	+65***	+36*	+88**	+48**
			Week 26			
ALP	-11	+14	+28*	+6	+39*	+47*
LALP	+64	+150***	+271***	+275	+175	+550***
IALP	NC	NC	NC	NC	NC	NC
CHOL	-17*	-24**	-28***	-10	-19*	-33***
TRG	NC	+26	+109***	+17	+37	+61

L=low-dose M=mid-dose H=high-dose NC=no change

ALP=alkaline phosphatase activity in the plasma
LALP= Liver ALP isoenzyme IALP=Intestinal ALP isoenzyme
CHOL=cholesterol TRG=triglycerides
*=p<0.05 **p<0.01 ***p<0.001

Urinalysis: In the first study, the quantitative analysis of overnight urine samples collected during week four did not reveal any inter-group differences. In the long-term study (sampling at week 25), a few decreases observed in the high-dose males (volume 35%, Na 31%, and chloride 32%) and females (potassium 20%, Ca 20%, and inorganic phosphorus 33%) were not observed after the recovery.

Gross pathology: In the 4-week study, except for the scab-formation, macroscopic examination did not reveal any treatment-related lesions. No macroscopic lesions were observed in the long-term study after the recovery period.

Organ weights: In both studies, the absolute and relative (to body) weights of the following organs were determined: adrenals, brain, epididymides, heart, kidneys, liver, lungs, ovaries, pituitary, prostate, salivary glands, seminal vesicles, spleen, testes, thymus, thyroid with parathyroid, and uterus with cervix.

In the first study, no inter-group differences in the organ weights were observed. In the long-term study, a slight increase in the absolute (13%) and relative (12%) liver weights in the high-dose males was not observed at the end of the recovery period.

Histopathology: Adequate Battery: yes
Peer review: Yes

Approximately 43 tissues/organs were examined microscopically. In addition, left adrenal (cortex and medulla), liver, femur (growth plate and dense bone), and treated skin were also subjected to electron microscopy examination.

In the 4-week study, the thickening of the stratum granulosum of the epidermis at the application site was observed in the mid- and high-dose rats. In the long-term study, acanthosis on the application sites was seen in all the high-dose, and 3/10 mid-dose males, and 3/10 low-dose females. No such lesions were observed after eight weeks of recovery.

Toxicokinetics: In the short-term study, the plasma drug concentration ratios on days 1, 7, and 24 indicated a non-linear increase. For instance, at the high dose level the values were 30-66% lower than predicted from the linear kinetics. The accumulation ratios generally close to unity indicated that

there was little or no drug accumulation after daily dermal applications. No sex differences in toxicokinetic behavior were observed.

In the 26-week study, the mean plasma adapalene levels at 24 hours post application on day 1, and weeks 4, 13, and 26 increased with the dose, however, the change was not dose-related. Overall, the values at the high-dose levels were approximately 65% lower than the predicted linear kinetics. Accordingly, a topical dose above 0.6mg/kg/day was considered likely to result in a disproportionately lower systemic exposure than would be predicted from a linear relationship.

The mean plasma drug level in the high-dose males was higher (35-55%), however, in females it was higher (10-73%) at the low-dose level. No significant differences between the two sexes were observed at the mid-dose level. In general, compared to day 1, the plasma drug levels were higher in weeks 4, 13, and 26. The drug concentration did not increase with the time of treatment.

Study title: 3. Adapalene and adapalene gel toxicity study by oral capsule and cutaneous administration to beagle dogs for 26 weeks.

Key study findings: No drug related systemic effects on bones were observed in dogs treated orally or topically. A dose-related epidermal hyperplasia and superficial dermal inflammation were observed in all the topically treated animals.

Study #: RDS.03.SPR.12225
Volume #, and page #: 28, 08
Conducting laboratory and location: [redacted]

Date of study initiation: 12 May 2000
GLP compliance: Yes
QA reports: yes
Drug lot #, and % purity: PA271G0118, [redacted]%

Methods:

Dose: Dermal: 0.1% and 0.3% adapalene gels on pre-shaved sites
Oral: Placebo containing triurate+lactose and 0.1 and 1.0mg adapalene in gelatin capsule/kg/day
Species/strain: beagle dogs
Number/sex/group or time point (main study): 4/sex/group
Route, formulation, volume, and infusion rate: topical gel (2mL/kg) and; one capsule/day
Satellite groups used for toxicokinetics or recovery: None
Age: 20-25 weeks
Weight: 6-10 kg

Sampling times: N/A
Unique study design or methodology: None

Observations and times: (the parameters are described under each endpoint under results.

Results: The objective of this study was to investigate the individual effects of topical and oral adapalene treatment on bones.

Mortality/Clinical signs: No drug-related deaths occurred during the treatment period. In all the treatment groups, dose-related signs such as reddening of ears, scrotum, or limbs were observed. These signs subsided by weeks 3-6 in-groups receiving oral doses, however, in the topical groups they increased as the study progressed.

In the topically treated groups, the dermal reactions consisted of red spots, flaking, and slight to well defined erythema. The incidence of these lesions increased with time, gradually involving all dogs during the last four months of treatment.

Body weights/Food consumption: The body weights and food consumption were determined daily.

Significant reduction (39% at $p<0.05$) in body weight was recorded in females treated with 0.3% adapalene gel, however, the corresponding reduction (5%) in food consumption was insignificant.

Ophthalmoscopy, EKG, Hematology, Clinical chemistry, and Urinalysis: Not performed.

Gross pathology: At the end of study period, all dogs were subjected to necropsy examination.

The Macroscopic lesions included flaking of skin, patchy congestion and occasional scabs in dogs treated topically.

Organ weights: Not determined

Histopathology: The following tissues were examined in all the animals: brain, cranium, femur, forelimbs, meninges, spinal column, and sternum. In addition, the skin on the application sites was also examined in the topically treated dogs.

No drug-related systemic effects on bones were observed in dogs treated orally or topically. A dose-related epidermal hyperplasia and superficial

dermal inflammation on the application sites was observed in all the gel treated animals.

Study title: 4. Adapalene toxicity study by oral capsule administration to beagle dogs for 26 weeks followed by an 8-week recovery period.

Key study findings: In dogs, the chronic oral administration of adapalene was associated with a significant increase in the activity of plasma alkaline phosphatase (ALP) and the liver specific alkaline phosphatase isoenzyme. The changes in bones due to increased osteoclastic activity and resorption resembled hypervitaminosis A syndrome. The increase in ALP activity was most probably due to the drug effect on bones. The effect on cranial bones is considered to be an extended pharmacological action of adapalene. The NOEL for oral adapalene was below 1mg/kg/day, the lowest dose tested.

Study no.: 1.CG.03.SRE.12076
Volume #, and page #: 29, 0011
Conducting laboratory and location: [ ]

Date of study initiation: 28 April 1998
GLP compliance: Yes
QA report: Yes
Drug, lot #, and % purity: Adapalene, PA271E0097, [ ]%

Methods

Doses: 0.0, 1.0, 4.5 and 20mg/kg/day
Species/strain: beagle dogs
Number/sex/group or time point (main study): 6/sex/group
Route, formulation, volume, and infusion rate: oral, adapalene, triurate and lactose in gelatin capsule
Satellite groups used for toxicokinetics or recovery: Two dogs from each group underwent a subsequent 8-week recovery period.
Age: 21-24 weeks
Weight: 8-12kg
Sampling times: The fasting blood samples for hematology and clinical chemistry were obtained prior to study initiation, and prior to dosing during weeks 4 and 26. Samples for toxicokinetics were drawn on day 1 prior to dosing, and during weeks 4 and 26 at 1, 2, 4, 8, and 24 post-dose hours.

Unique study design or methodology (if any): None

Observations and times: (the parameters are described under each endpoint under results.

Results

Mortality/Clinical signs: Animals were examined daily for clinical signs of toxicity, morbidity and mortality.

No deaths directly connected to the drug treatment occurred. One high-dose male and one high-dose female were killed on humane grounds during week 10 and week 14, respectively. These deaths resulted from histologically confirmed narcotizing polyarteritis, a spontaneous condition in beagle dogs that was possibly exacerbated by drug treatment. The drug-related sign of thin body build was restricted to one low-dose male, two males and one female from the mid-dose group, and one high-dose male. This condition persisted throughout the treatment period. In addition, one high-dose female also exhibited convulsions, ataxia, underactivity and salivation in week 25.

Body weights/Food consumption: A slight but statistically insignificant decrease (4-10%) in body weights was recorded in both the sexes at the end of the treatment period. At the end of recovery period, this insignificant decrease ranged between 2-12 percent. No parallel changes in food consumption were observed at any stage of the study period.

Ophthalmoscopy: The examinations held prior to study initiation and at the end of treatment did not reveal any drug-related changes.

EKG: The electrocardiograms obtained at 3 and 24 hours post-dose in week 13 and just before the end of the treatment period did not reveal any abnormalities. Similarly, blood pressure and pulse rates were not affected by the treatment.

Hematology: No dose-related changes were recorded. Some statistically significant changes in a few parameters were randomly distributed, and therefore, were not considered to be of any biological significance.

Clinical chemistry: The total plasma alkaline phosphatase and liver alkaline phosphatase isoenzyme activities in the high-dose males at week 4 (56 and 53%, respectively at $p<0.05$) and week 26 (58 and 67%, respectively at $p<0.01$) were greater than controls. These activities in the high-dose females were increased at week 26 (77 and 87%, respectively at $p<0.01$). The activity of plasma aspartate amino-transferase in the mid- (35% at $p<0.05$) and high-dose females at week 4 and 26 (61 and 50%, respectively at $p< 0.001$) and high-dose males at week 26 (48% at $p<0.05$) was also increased. No inter-group differences in these enzyme activities were observed after the recovery period.

Urinalysis: The urine samples collected at week 25 did not reveal any drug-related changes in the whole spectrum of evaluated parameters.

Gross pathology: Drug-related macroscopic lesions included excess of fluid around the brain with dilated lateral ventricles, fluid surrounding the spinal cord, thickened meninges, thin spinal column (females only), musculo-skeletal abnormalities, and skin encrustation (Table 1). Some of these lesions persisted during the recovery period.

Table 1. Summary of gross pathologic findings in dogs sacrificed after 26 weeks of treatment.

	MALES[n]			FEMALES[n]		
Lesion	Low	Mid	High	Low	Mid	High
Brain excess fluid around	1*	1	2	2	4	2
Meninges thickened	0	0	3	0	2	3
Spinal c.Cerv excess fluid	0	0	0	0	1	2
LN Thymic dark	0	1	3	0	1	1
Musculo-skeletal abnormal shape	0	1	1	0	1	3
Skin encrustation	0	2	0	0	0	3

*Number of animals with lesion
n= low- and mid-dose 4, high-dose 3

Organ weights: Only the mean relative (to body) weights of liver in the high-dose males (+29% at $p<0.05$) and females (+42% at $p<0.05$) were increased in dogs sacrificed after 26 weeks of treatment. The mean relative kidney weight in high dose females was also increased (+41% at $p<0.01$). No inter-group differences were observed in these weights after the recovery. No other organs were affected.

Histopathology: The following tissues were examined microscopically: adrenals, brain, femur, heart, kidneys, liver, lungs, mammary area, spinal cord, sternum, stomach, thyroid, and uterus.

The microscopic examination of multiple sections of brain did not reveal any pathological conditions that can be related to excess fluid and dilated ventricles.

Thickened meninges with acute and chronic inflammation, hyperplasia of the arachnoid epithelium and hemorrhage some time accompanied by

hemosiderin pigment, were observed in all the high-dose dogs and in two mid- and high-dose females (Table 2). The histopathological examination of a high-dose male killed on humane ground indicated focal hemorrhage in the inner meningeal membrane.

In the high-dose animals, the incidence of thin cranial bone with reduced Haversian canals observed at week 26 became more pronounced during the recovery period. In the sternum, the increased cellularity of marrow observed at week 26 was much diminished at recovery week 8.

Table 2. Summary of microscopic lesions at week 26 (*wk*) and recovery week 8 (*rwk*)

Lesion	MALES			FEMALES		
	L	M	H	L	M	H
Meninges						
Acute inflammation wk. 26	0	0	0	0	0	1/3
Chronic inflammation						
wk 26	1/1	0	3/3	0	2/2	2/3
rwk 8	1/1	0	1/1	0	1/1	0
Hemorrhage						
wk 26	0	0	3/3	0	2/2	1/3
rwk 8	1/1	0	1/1	0	1/1	0
Hemosiderin pigment						
wk 26	0	0	1/3	0	1/3	2/3
rwk 8	0	0	1/1	0	0	0
Hyperplasia of arachnoid epithelium						
wk 26	0	0	1/3	0	1/2	3/3
rwk 8	1/1	0	1/1	0	0	0
Cranium						
Thinned						
wk 26	0	1/1	1/1	0	1/1	3/3
rwk 8	2/2	2/2	2/2	1/2	2/2	2/2
Reduced Haversian canal						
wk 26	0	1/1	1/1	0	1/1	3/3
rwk 8	2/2	2/2	2/2	1/2	2/2	2/2
Sternum						
Reduced medullary cavity						
wk 26	0	0	3/3	0	0	3/3
rwk 8	0	0	0	0	0	0
Increased cellularity of marrow						
wk 26	0	0	2/3	0	2/4	2/3
rwk 8	0	0	0	0	0	1/2

Toxicokinetics: The plasma drug level at 24 hour post-dose was quantifiable in all dogs at day 1 and weeks 4 and 26, indicating a continuous exposure to adapalene throughout the study period (Table 3).

Table 3. Summary of toxicokinetic data.

	Day 1		Week 4		Week 26	
Dose group	Males	Females	Males	Females	Males	Females
Low Cmax	116	70	172	149	200	193
AUC_{24}	1550	1030	2120	1960	2480	2380
Mid Cmax	227	286	705	533	587	483
AUC_{24}	3120	3480	6640	4590	6160	4950
High Cmax	819	630	799	865	1310	1170
AUC_{24}	9410	5890	7040	6640	11400	9350

The average Tmax of around 4 hours (range 2-24 hours) was independent of dose and sex.

The values of Cmax and AUC_{24} increased ($p<0.001$). with time and dose, however, the increases were non-proportional to dose. Overall at the high-dose level, the values of Cmax and AUC_{24hr} were approximately 72% lower than those predicted from a linear relationship.

The mean accumulation ratios were generally greater than unity, indicating some drug accumulation after repeated oral administration. The similar accumulation ratios at weeks 4 and 26 indicated that a steady state was probably achieved at week 4. The terminal $T_{1/2}$ ranged from 2.3 to 10.5 hours.

The plasma drug concentrations in the recovery samples were below the detection limit of 1ng/mL.

2.6.6.4 Genetic toxicology: No new studies were reported.

2.6.6.5 Carcinogenicity: No additional studies were conducted. The protocols and findings of the previous studies were approved by the CAC. The data is included in the approved labels for various preparations of adapalene.

2.6.6.6 Reproductive and developmental toxicology: No additional studies were conducted.

2.6.6.7 Local tolerance: Adapalene 0.3% gel exhibited a very low acute irritation potential in a primary assay in NZW rabbits. In a 28-day topical study with 0.3% gel, rabbits exhibited a minimal to slight and diffused epidermal hyperplasia. In rabbit ocular irritation assay, 0.3% gel caused a very slight irritation in the conjunctiva.

In Magnusson and Klingman test in guinea pigs, adapalene did not cause any delayed contact hypersensitivity. In another assay, adapalene gel 0.1% did not exhibit any signs of phototoxicity or photoallergy.

2.6.6.8 Special toxicology studies: No new studies were conducted.

2.6.6.9 Discussion and Conclusions: See under the Overall Summary of Toxicology Studies.

2.6.6.10 Tables and Figures: N/A

2.6.7 TOXICOLOGY TABULATED SUMMARY

[pivotal studies pertinent to the primary indication and core pharmacology studies relevant to the primary pharmacodynamic effect, as available and as provided by the sponsor]

See summary under toxicology

OVERALL CONCLUSIONS AND RECOMMENDATIONS

Conclusions: To date, three formulations of adapalene (0.1% solution, 0.1% gel, and 0.1% cream) for the treatment of *acne vulgaris* have been approved for marketing in the United States. A large number of *in vivo* and *in vitro* non-clinical studies were conducted to support the safety of these drug products. The protocols for a majority of these studies had also included testing of these formulations at 0.3% strength of adapalene. Of a total of 26 studies conducted with 0.3% adapalene, 17 were conducted with various gel formulations. In the recent submission, 16 additional pharmacokinetic and general toxicology studies conducted with the proposed 0.3% adapalene gel formulation were reported.

In a 26-week oral pharmacokinetic study (0.15, 1.5, and 15mg/kg/day) in rats, the dose-dependent bioavailability for adapalene ranged between 3-75 percent. In the sub-chronic and chronic multi-species oral and intraperitoneal studies (1-500mg/kg/day), the systemic toxic effects (because of high exposure) such as loss of hairs, increased osteoclastic activity, spontaneous long bone fractures, and skeletal resorptions, resembled hypervitaminosis A syndrome. In fact, in dogs an oral dose of 1mg/kg/day was considered to be close to the threshold dose causing cranial bone changes.

In the multi-species dermal pharmacokinetic studies, irrespective of the formulation and drug concentration, the systemic absorption/bioavailability never exceeded 5% of the applied dose. The topical applications of 0.3% adapalene gel in rats at the maximum feasible dose of 2 mL/kg (6mg adapalene/kg/day) for 4-26 weeks did not cause any systemic toxicity. The dose-related scab formation and acanthosis disappeared during 8 weeks of recovery period. Dogs treated topically (6mg/kg/day) for 26 weeks did not exhibit any bone-related systemic toxicity, and the epidermal hyperplasia and superficial inflammation on the application sites were transient and non-severe in nature.

The maximum recommended human dose (MRHD) for 0.3% adapalene gel is 2.5g /subject of 60kg/day, or 4.6-mg adapalene/m^2/day. Taking into account the mouse dermal carcinogenicity study (the highest safe dose 4mg/kg/day), the margin of safety will be approximately 3 times the MRHD in terms of body surface area. For the rat and rabbit dermal studies conducted at the maximum feasible dose (6mg/kg/day), the margin of safety values will be approximately 8 and 16, respectively. However, these calculated values are based on the assumption of 100% systemic absorption, whereas, the actual absorption in animals did not exceed 5 percent. Therefore, the realistic margin of safety in each case will be much greater. This fact is also supported by some human data.

1. No drug was detected in the plasma of women treated topically with 2g of 0.1% adapalene gel per day for 3 months.

2. The absorption studies with human skin preparations or cultures revealed a slightly higher absorption (never exceeding 10%) than in majority of the animals.

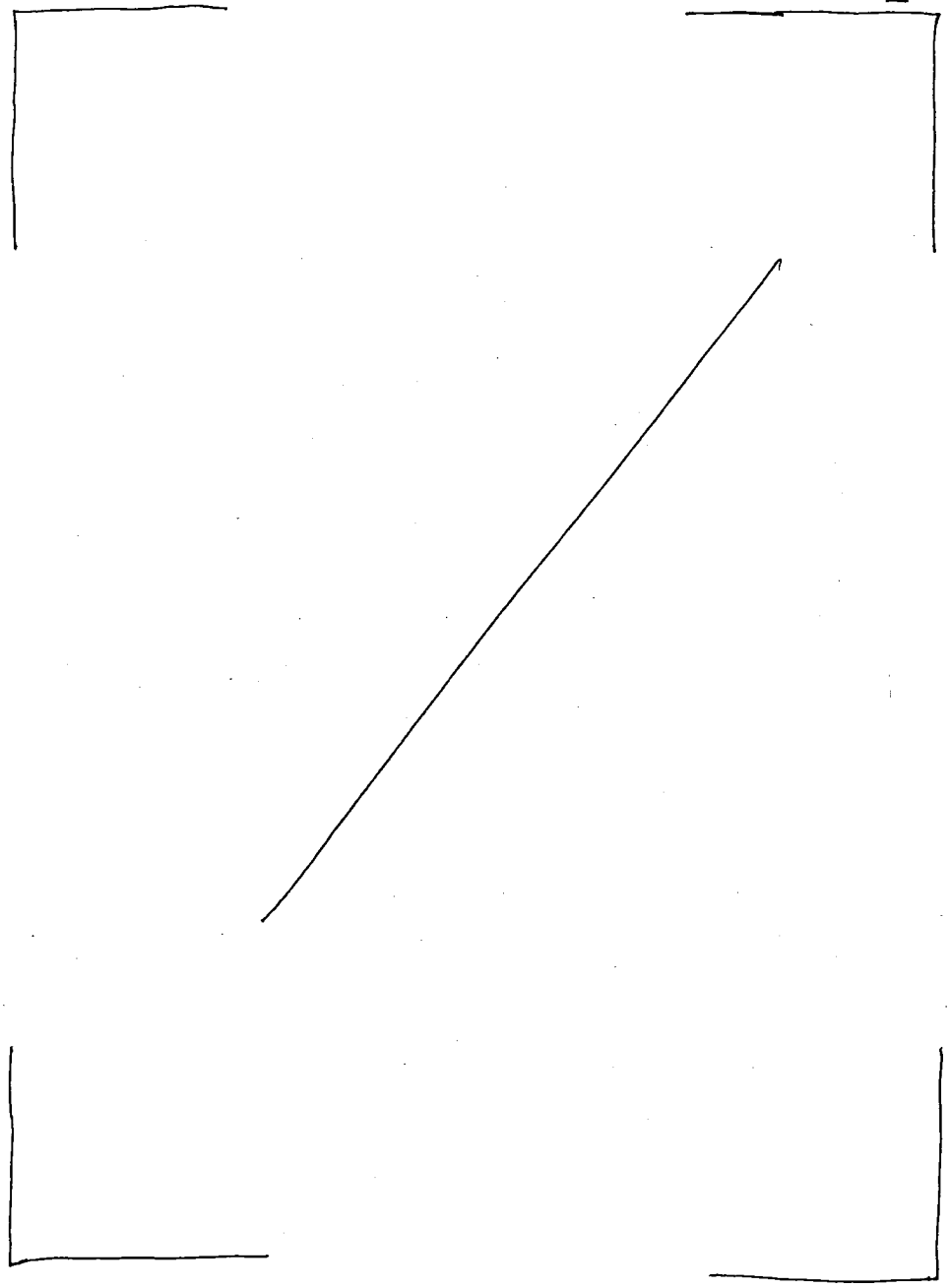
3. In four volunteers, the total amount of radioactivity found in the feces following a topical application of 0.1% adapalene solution amounted to 0.02-0.06% of the applied dose. No significant amount of radioactivity was found in the urine.

Unresolved toxicology issues (if any): None

Recommendations: I have no objection to the approval of this New Drug Application.

Suggested labeling:

Carcinogenesis, Mutagenesis, Impairment of Fertility: [ ]



Signatures (optional):

Reviewer Signature _____

Supervisor Signature_____ Concurrence Yes ___ No ___

APPENDIX/ATTACHMENTS

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/s/

Kumar Mainigi
11/23/04 10:38:09 AM
PHARMACOLOGIST

Paul Brown
11/24/04 11:38:23 AM
PHARMACOLOGIST

Pharmacology/Toxicology Memorandum

NDA 21-753
Drug: Adapalene 0.3% Gel
Sponsor: Galderma Laboratories
Re: Comparison of animal teratogenicity data with human exposure
To: Jonathan Wilkin, M.D., Division Director
From: Paul Brown, Ph.D., Pharmacology/Toxicology Supervisor
Division of Dermatologic and Dental Drug Products
HFD 540
Date of review: January 21, 2005

Introduction:
This memorandum provides pharm/tox comment on the animal teratogenicity data and the recently reviewed human pharmacokinetic data for the 0.3% adapalene gel.

Discussion:
Maximum human AUC_{0-24} according to the maximal human use PK study was 36.1 ng·h/mL and the maximum C_{max} was 2 ng/mL.

Note: The animal AUC data presented below for the topical studies are not from the teratogenicity studies but are from studies using the same species and dose.
Outcome: + = teratogenic, - = not teratogenic

Rat dermal mg/kg	Outcome	Fold human mg/m^2	AUC_{0-24} (ng·h/mL)	Fold human AUC
0.6	-			
2	-			
6	-(minimal increase in supernumerary ribs)	8	204	5.7

Rabbit dermal mg/kg	Outcome	Fold human mg/m^2	AUC_{0-24} (ng·h/mL)	Fold human AUC
0.6	-			
2	-			
6	-	16	1036	28.7

Using an AUC comparison of animal to human exposure instead of mg/m^2 comparison of total dose does not change the margin of safety much for the dermal studies.

Note: Adequate data for the oral AUC in the rat has not been located. The AUC data in the table below was from a separate study in rats that examined the pharmacokinetics of a 0.5 mg/kg oral dose.

Rat oral mg/kg	Outcome	Fold human mg/m^2	AUC_{0-24} (ng·h/mL)	Fold human AUC
5	-	7	? (0.5mg/kg AUC=196)	?
25	+	33		
60	+			

Good data for the oral AUC in rat has not been located. However, it appears that an AUC comparison for this study would provide a larger margin of safety than the mg/m^2 comparison.

Conclusions:
1. Adapalene is teratogenic when given to animals at sufficient doses.
2. The NOAEL for teratogenesis in humans for adapalene is unknown. With some other retinoids the human appears to be a sensitive species.
3. The 0.1% adapalene product is currently labeled with Pregnancy category C. Previous pharmacokinetic studies with the 0.1% gel showed that levels of adapalene in patients were less than 0.35 ng/mL. Therefore, the 0.3% gel appears to produce higher exposures than the 0.1% gel and would have a commensurate increased teratogenic risk.
4. The indication for 0.3% adapalene is acne vulgaris; therefore, the patient population is vulnerable for teratogenicity.

Recommendation:
Because of the potential teratogenic risk, if the 0.3% adapalene gel were approved for acne vulgaris, it would not be appropriate to use the drug in pregnant women. [ ]

Suggested nonclinical wording for the relevant sections of the labeling:
(This differs substantially from the labeling of the 0.1% gel. This labeling is consistent with other topical non-endogenous retinoids with measurable systemic exposure. [ ]

2 page(s) of draft labeling has been removed from this portion of the review.

Pharmacology Memorandum - 1/21/05

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/s/

Paul Brown
1/21/05 05:34:32 PM
PHARMACOLOGIST

[ ]

Jonathan Wilkin
1/31/05 12:15:41 PM
MEDICAL OFFICER

Pharmacology/Toxicology Memorandum

NDA 21-753
Drug: Adapalene 0.3% Gel
Sponsor: Galderma Laboratories
Submission: letter date 18 December 2006 / AZ

Reviewer: Paul Brown, Ph.D., Pharmacology/Toxicology Supervisor, Division of Dermatology and Dental Products
Date of review: June 1, 2007

Introduction:
This submission is a response to the Not Approvable letter dated 1 February 2005. The sponsor has provided a safety update and revised labeling.

The sponsor notes that seven additional nonclinical studies have been completed since submission of the 4-month safety update for the NDA dated August 16, 2004. Three of these studies were repeat dose dermal toxicology studies in minipigs with adapalene 0.1%/benzoyl peroxide 2.5% gel. Reports of these studies were submitted to IND 67,801 (SN 012). These studies were reviewed and no new unexpected toxicity findings were noted.

The following four new in vitro liberation-penetration studies were also completed.
1. Comparison of in vitro liberation-penetration of [^{14}C]-adapalene formulated in seven different formulations through human skin. Study RDS.03.RDE.4777
2. Comparison of the in vitro liberation-penetration of adapalene formulated in four formulations through human skin. Study RDS.03.RDE.4762
3. In vitro percutaneous absorption of adapalene through human skin membranes using static diffusion cells. Study RDS.03.RDE.4769
4. Comparison of in vitro liberation-penetration of adapalene (0.1% w/w) and benzoyl peroxide 2.5% (w/w) formulated in different formulations through human skin. Study RDS.03.RDE.4781

These studies all examined the absorption of adapalene from various formulations through human skin in vitro. Only the first study used a 0.3% adapalene gel. The results of the study suggest that adapalene absorption was greater from gel formulations than lotion formulations and that absorption was greater from 0.3% formulations than 0.1% formulations. These data are not required or relevant for labeling since in vivo human absorption information is available for adapalene 0.3% gel and this information is already incorporated into the labeling. These studies will not be further reviewed here.

Comments on labeling:
The sponsor's proposed wording for the *Carcinogenesis, Mutagenesis, Impairment of Fertility* and the *Pregnancy* sections are shown below. This wording differs significantly from that in the original submission to the NDA and differs significantly from the approved Differin products. Suggested deletions are shown as strikeout text and additions are

highlighted. Recommended changes include several corrections to the animal to human dose ratios. Calculations for these dose ratios are shown at the end of this review. Other changes include eliminating some of the redundant animal data in the *Pregnancy* section and addition of some information about specific teratogenic findings. The following sentence was added to the *Pregnancy* section to address the suggestion from the Pediatric and Maternal Health Staff to include information on the general teratogenic risk of retinoids: "Retinoids may cause fetal harm, when administered to pregnant women."

In addition, it would be appropriate to add further information describing the findings of the oral segment II (embryofetal toxicity) studies in the animal data section of the labeling. This information may be useful to patients and health care professionals when considering the risks of using this drug during pregnancy. Similar information is included in the labeling of other drug products. The following sentence could be inserted into the animal data section after the sentence noting that the drug was teratogenic after oral administration: Findings included cleft palate, micropthalmia, encephalocele and skeletal abnormalities in the rat and umbilical hernia, exopthalmos and kidney and skeletal abnormalities in the rabbit.

With these changes, the labeling appears to be a reasonable presentation of the information.

Suggested labeling with changes marked:
Carcinogenesis, Mutagenesis, Impairment of Fertility: Carcinogenicity studies with adapalene have been conducted in mice at topical doses of 0.4, 1.3, and 4.0 mg/kg/day, and in rats at oral doses of 0.15, 0.5, and 1.5 mg/kg/day. These doses are up to ~~/~~3 times (mice) and 2 times (rats) in terms of mg/m²/day the potential exposure at the maximum recommended human dose (MRHD), assumed to be 2.5 grams DIFFERIN Gel, 0.3%. In the oral study, increased incidence of benign and malignant pheochromocytomas in the adrenal medullas of male rats was observed.

No photocarcinogenicity studies were conducted. Animal studies have shown an increased risk of skin neoplasms with the use of pharmacologically similar drugs (e.g., retinoids) when exposed to UV irradiation in the laboratory or to sunlight. Although the significance of these studies to human use is not clear, patients should be advised to avoid or minimize exposure to either sunlight or artificial UV irradiation sources.

Adapalene did not exhibit mutagenic or genotoxic effects *in vitro* (Ames test, Chinese hamster ovary cell assay, mouse lymphoma TK assay) and *in vivo* (mouse micronucleous test).

Reproductive function and fertility studies were conducted in rats administered oral doses of adapalene in amounts up to 20 mg/kg/day (up to ~~/~~ 26 times the MRHD based on mg/m² comparisons). No effects of adapalene were found on the reproductive performance or fertility of the F_0 males or females. There were also no detectable effects on the growth, development and subsequent reproductive function of the F_1 offspring.

***Pregnancy*: Teratogenic effects. Pregnancy Category C.**
Retinoids may cause fetal harm, when administered to pregnant women. Adapalene has been shown to be teratogenic in rats and rabbits when administered orally (see Animal Data below).

There are no adequate and well-controlled studies in pregnant women. DIFFERIN Gel, 0.3% should be used during pregnancy only if the potential benefit justifies the potential risk to the fetus.

1. Human Data

In clinical trials involving DIFFERIN Gel, 0.3% in the treatment of acne vulgaris, women of child-bearing potential initiated treatment only after having had a negative pregnancy test and used effective birth control measures during therapy. However, 6 women treated with DIFFERIN Gel, 0.3% became pregnant. One patient elected to terminate the pregnancy, two patients delivered healthy babies by normal delivery, two patients delivered prematurely and the babies remained in intensive care until reaching a healthy state and one patient was lost to follow-up.

2. Animal Data

- No teratogenic effects were seen in rats at oral doses of 0.15 to 5.0 mg/kg/day adapalene representing up to / 6 times the maximum recommended human dose (MRHD) based on mg/m^2 comparisons. Adapalene has been shown to be teratogenic in rats and rabbits when administered orally at doses ≥ 25 mg/kg representing / 32 and 65 times, respectively, the MRHD based on mg/m^2 comparisons. Findings included cleft palate, microphthalmia, encephalocele and skeletal abnormalities in the rat and umbilical hernia, exophthalmos and kidney and skeletal abnormalities in the rabbit.

- Cutaneous teratology studies in rats and rabbits at doses of 0.6, 2.0, and 6.0 mg/kg/day exhibited no fetotoxicity and only minimal increases in supernumerary ribs in both species and delayed ossification in rabbits. Systemic exposure (AUC0-24h) to adapalene 0.3% gel at topical doses of 6.0 mg/kg/day in rats and rabbits represented 5.7 and 28.7 times, respectively, the exposure in acne patients treated with adapalene 0.3% gel applied to the face, chest and back (2 grams applied to 1000 cm^2 of acne involved skin).

All pregnancies have a risk of birth defect, loss, or other adverse event regardless of drug exposure. Typically, estimates of increased fetal risk from drug exposure rely heavily on animal data. However, animal studies do not always predict effects in humans. Even if human data are available, such data may not be sufficient to determine whether there is an increased risk to the fetus. Drug effects on behavior, cognitive function, and fertility in the offspring are particularly difficult to assess.

Dose calculations for Differin 0.3% gel used in labeling:
Human dose of 2.5 g gel in 60 kg individual using Km of 37:
2.5 g = 2500 mg
2500 mg x 0.3% = 2500 mg x 0.003 = 7.5 mg
7.5 mg ÷ 60 kg = 0.125 mg/kg
0.125 x 37 = 4.625 mg adapalene/m^2

Species	Dose (mg/kg)	Km	Dose (mg/m^2)	Animal/human
Mouse	4	3	12	3
Rat	1.5	6	9	2
Rat	5	6	30	6
Rat	20	6	120	26
Rat	25	6	150	32
Rabbit	25	12	300	65

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/s/

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