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Volume # 1, and page # 1
Conducting laboratory and location: [redacted]
Date of study initiation: 8-18-1993
GLP compliance: Yes
QA report: yes (X) no ( )
Drug, lot #, and % purity: Admixture Active Gel (benzoyl peroxide 5% and clindamycin phosphate 1%), 3E225D229, (111% and 116%); 3E344K342, (100% and 118%); 3M322B328, (100% and 119%); 3M322C252, (98.2% and 117%); 3M322C252A, (105% and 115%). Admixture Placebo Gel, 3G218D230, 3K345K343, 3M321M318B, 3M321C253, and 4E646X253.
CAC concurrence: NA

Methods

Doses: Benzoyl peroxide/clindamycin phosphate, 0/0, 250/50, and 500/100 mg/kg/day

Basis of dose selection (MTD, MFD, AUC etc.): The doses to be used in the 52 week photococarcinogenicity study were determined from the results of 1 week and 8 week range-finding and tolerance studies.

In the one week dose range finding study, mice were treated topically with a single dose of up to 500/100 mg/kg benzoyl peroxide/clindamycin phosphate (0.025, 0.05, 0.1, or 0.2 mL gel). Some animals were also treated with 0.2 mL of the placebo gel or were left untreated. Treated and untreated sites were then irradiated with up to 2.7 times the mouse MED using a Berger compact arc high intensity solar simulator with a 1 mm WG320 Schott glass filter. Mice were examined for signs of inflammation of the irradiated site at approximately 24, 48 and 72 hours after irradiation. Neither the placebo nor the clindamycin gel enhanced nor inhibited the acute effects of the light on the skin of the mice. Therefore, 0.05, 0.1, and 0.2 mL were used in the 8 week tolerance study.

In the 8 week tolerance study, mice were treated topically 5 days per week with up to 500/100 mg/kg/day benzoyl peroxide/clindamycin phosphate (0.05, 0.1, or 0.2 mL gel). Some animals were also treated with 0.2 mL of the placebo gel or were left untreated. Treated and untreated animals were then irradiated with 120 RBU of solar simulated light from a 6.5 kilowatt xenon arc filtered with a 1 mm WG320 Schott glass filter. Animals were irradiated on the same five days per week that they were treated with drug or placebo. The test articles were applied after the daily UV exposure on Monday, Wednesday, and Friday and before daily UV exposure on Tuesday and Thursday. Some animals were also treated with the test articles and not irradiated. Erythema (Grade 1 or 2), edema (Grade 1), flaking (Grade 1 or 2) were noted in mice treated with Admixture Active Gel, but this is not dose-dependent. Skin thickening (Grade 1) was seen in all groups. There were no differences in skin reactions between the males and females and between with and without UVR exposure. All clinical findings other than skin reactions were considered unrelated to the test articles. The 0.1 and 0.2 mL Admixture Active Gel were selected for the 52 week photocarcinogenicity study.

Study Design Table (main study):

Group	Test article	Volume of test article applied (mL/mouse)	Dose of benzoyl peroxide/clindamycin phosphate (mg/kg/day)	RBU/week
1	Untreated	0	0/0	600
2	Untreated	0	0/0	1200
3	Admixture Placebo gel	0.2	0/0	600
4	Admixture Active Gel	0.1	250/50	600
5	Admixture Active Gel	0.2	500/100	600
6	Benzoyl Peroxide 5% Gel	0.2	500/0	600
7	Clindamycin Phosphate 1% Gel	0.2	0/100	600

Species/strain: Albino hairless mice — SKH1(hr/hr)BR

Number/sex/group (main study): 36/sex/group

Route, formulation, volume: The test articles (0, 100, or 200 µL) were applied topically to an approximately 20 cm^2 area of skin on the backs of the animals.

Formulations:

Ingredient	% w/w	
	Admixture Active Gel	Placebo Gel
Clindamycin phosphate	1.000	0
Benzoyl peroxide	—	0
—	—	—
Propylene glycol, USP	—	—
Carbomer — USP	—	—
Potassium hydroxide	—	—
Purified water, USP	—	—

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Note: This formulation is different from that proposed for clinical use.

Frequency of dosing: Once daily Monday through Friday. On Monday, Wednesday, and Friday test article was applied 1 hr after irradiation and on Tuesdays and Thursdays test article was applied 1 hr before irradiation. Treatment was continued for 40 weeks.

Satellite groups used for toxicokinetics or special groups: None

Age: 5 Weeks

Animal housing: Individual stainless steel cages

Restriction paradigm for dietary restriction studies: NA

Dual controls employed: Yes

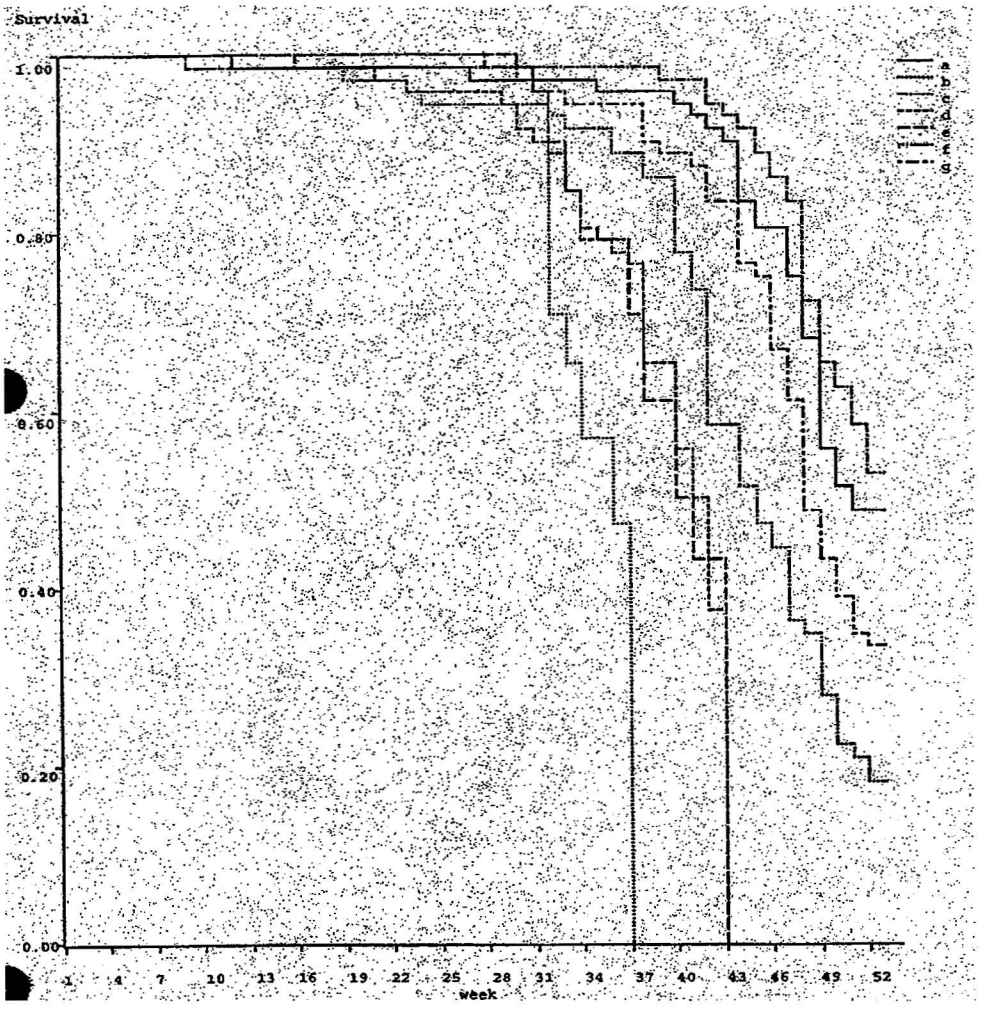
Interim sacrifices: Mice found dead were necropsied. Mice *in extremis* and mice with tumors greater than 10 mm planar diameter were sacrificed and necropsied. All mice in a dosage group were sacrificed if less than one-half of the mice in the group survived and more than one-half of the surviving mice in the group had tumors of at least 4 mm planar diameter.

Deviations from original study protocol: No significant changes.

Observation times and results:

Mortality: Mice were observed for viability twice daily.

The figure below shows survival of the animals throughout the 52 weeks of the study. The letters a through g correspond to the group numbers 1 to 7. The untreated group receiving 600 RBU/week was Group a, the untreated group receiving 1200 RBU/week was Group b, the placebo gel group was Group c, and the Admixture Active Gel 0.1 and 0.2 mL were Groups d and e. It can be seen that the high dose of radiation (b) produced the largest decrease in survival. The survival of the Admixture Placebo Gel-treated animals (c) was slightly higher than that of the untreated animals receiving 600 RBU/week (a). Treatment with Admixture Active Gel (d and e) caused significant decreases in animal survival compared to the placebo (c) or untreated animals (a) receiving 600 RBU/week. Most of the deaths were because of early sacrifice due to tumor burden.



Clinical signs: Clinical signs were evaluated at least once a week.

No treatment related clinical findings were noted other than the skin reactions. The placebo gel did not produce any increase in skin reactions when compared to the untreated animals. Greater incidences of erythema, flaking, and thickening of the skin (in a dose-dependent fashion) were observed in male and female mice treated with Admixture Active Gel compared to the placebo-treated animals receiving 600 RBU/week. Edema was seen in the test article-treated animals, not in the untreated groups receiving 600 RBU/week. The severity of these skin reactions was greater in the male mice than in female mice. The skin reactions observed in Admixture Active Gel-treated mice generally did not exceed the incidence and severity of reactions observed in the untreated mice receiving 1200 RBU/week.

Body weights: Body weights were recorded weekly.

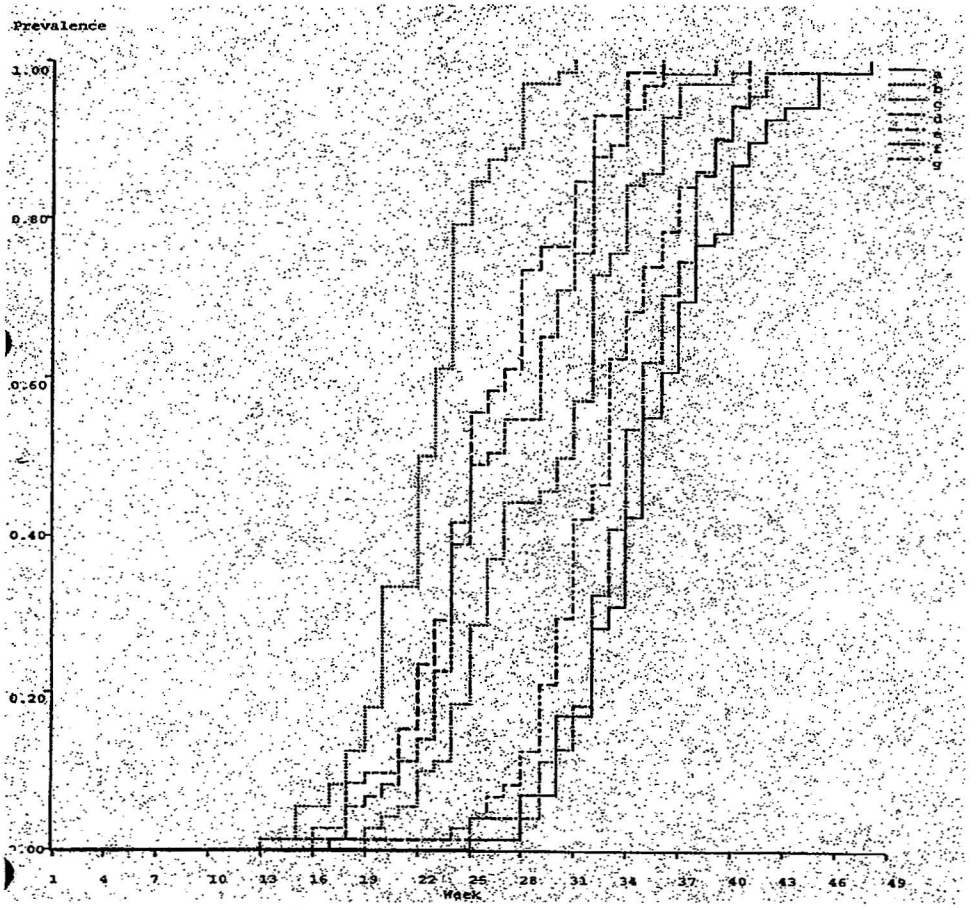
Admixture Active Gel or Placebo Gel treatment did not produce any significant effects on final body weight or body weight gain.

Gross pathology: All mice, whether found dead, euthanized *in extremis* or sacrificed at the end of the study were subjected to a gross examination of the external surfaces of the body and all internal tissues.

The groups treated with Admixture Active Gel had a slightly increased incidence of enlarged and mottled livers which might be related to the increased skin reaction and tumor burden in these groups.

Skin tumor: Each mouse was examined weekly for tumor development.

Topical application of Admixture Active Gel (Groups 4 and 5) was associated with apparent enhancement of photocarcinogenesis. The figure below is for perceptible tumors (sex combined), but figures for other tumor sizes are similar. The untreated group receiving 600 RBU/week was Group a, the untreated group receiving 1200 RBU/week was Group b, the placebo gel group was Group c, and the Admixture Active Gel 0.1 and 0.2 mL were Groups d and e. Comparison of Groups a and b demonstrated a UVR dose-dependent response for all tumor size categories. The placebo gel (c) appeared to cause a very slight, not statistically significant enhancement of UV induced skin tumors, as compared to the untreated group (a) receiving 600 RBU/week. The tumor prevalence in Groups d and e mice was increased for all tumor size categories, as compared with Group a or c mice. Within Groups d and e, the tumor prevalence was increased for the males as compared to the females, indicating a gender difference in tumor responses.



The table below shows the median time to tumor onset for tumors ≥1 mm in each group. It can be seen that the time to tumor onset in Admixture Active Gel-treated groups (Groups 4 and 5) was shorter than that in the untreated group receiving 600 RBU/week of UV radiation (Group 1) or the placebo gel group (Group 3).

Unbiased Median Tumor Onset for Tumors ≥1 mm[a]

Group	1	2	3	4	5	6	7
Test Article Dosage (mg/kg)	None	None	0 Placebo	250/50 BPO/CL	500/100 BPO/CL	500 BPO	100 CL
UVR Exposure (RBU/Week)	600	1200	600	600	600	600	600
Sexes Combined Median (Weeks)	36.00	24.00	35.00	30.00	28.00	33.00	34.00
Males Median (Weeks)	36.00	26.00	35.50	27.00	28.00	30.50	34.50
Females Median (Weeks)	38.00	24.00	35.00	32.00	30.50	34.00	34.00

The tables below show statistical comparisons of the tumor onset data for tumors ≥1 mm in each group (sexes combined). Results for other tumor size categories were similar. It can be seen that the time to tumor onset was significantly earlier in Group 2 than in Group 1 and other test article-treated groups (Groups 3, 4, 5, 6, and 7). The time to tumor onset for the placebo gel treated group (Group 3) was not significantly different from that for Groups 1. Topical treatment of Admixture Active Gel (Groups 4 and 5) was associated with significant (P<0.001) acceleration in tumor onset as compared to Group 1 or 3. Males tended to be significantly more affected than females.

Group Comparisons (Peto Analysis) of Tumor Onset for Tumors ≥1 mm[a]

Sexes Combined

Group	1	2	3	4	5	6	7
Test Article Dosage (mg/kg)	None	None	0 Placebo	250/50 BPO/CL	500/100 BPO/CL	500 BPO	100 CL
UVR Exposure (RBU/Week)	600	1200	600	600	600	600	600
	C	+++	N.S.	+++	+++	+++	+
		C	---	---	---	---	---
			C	+++	+++	+++	N.S.
				C	N.S.	-	---
					C	---	---
						C	---

Codes relate to level of statistical significance based on two-tailed p-values. Note that the comparison group is indicated by a C on the same line. Plus signs indicate the group specified has a risk greater than that with which it is being compared; minus signs indicate the group specified has a risk less than that with which it is being compared.

+++, --- = P<0.001
++, -- = P<0.01
+, - = P<0.05
N.S. = Not Significant

4. As stated in the review by Dr. Paul Brown in NDA 50-741 (Duac Gel), "The carcinogenicity of benzoyl peroxide has been investigated in a number of published studies; however, most of the studies have not been of two years duration and have not used daily application. In most studies, benzoyl peroxide applied alone did not produce skin tumors; however, in a study using SENCAR mice, benzoyl peroxide alone applied 2 times per week for 51 weeks produced skin tumors in 5 of 20 animals (Kurokawa et al., 1984). While the studies evaluating the carcinogenicity of benzoyl peroxide applied alone are limited, the studies clearly show that benzoyl peroxide is a tumor promoter and tumor progression agent in the skin in several animal models. The models in which benzoyl peroxide has shown activity include chemically or UV-initiated mice and hamsters. In one study conducted by the National Toxicology Program, benzoyl peroxide was shown to promote tumor formation initiated by dimethylbenzathracene or methyl-nitro-nitrosoguanidine in B6C3F$_1$, Swiss CD-1 and SENCAR mice (NTP TR 441, 1996). In this study the initiator was administered once and benzoyl peroxide was administered weekly for 52 weeks. Benzoyl peroxide by itself did not produce skin tumors in any strain in this study." "The Tg.AC transgenic mouse model is considered by the Agency

to be an acceptable alternative model for the evaluation of carcinogenicity. Studies with Tg.AC mice are typically conducted using 20 weeks of treatment and compounds that are tumor promoters can be detected by this model. Benzoyl peroxide in acetone at doses of 5 and 10 mg administered twice per week induced skin tumors in Tg.AC mice in a study with a total of 20 weeks of topical treatment (Spalding et al., 1993)."

The following wording in the labeling of some approved drug products containing benzoyl peroxide, "Benzoyl peroxide has been shown to be a tumor promoter and progression agent in a number of animal studies" and "Benzoyl peroxide in acetone at doses of 5 and 10 mg administered twice per week induced skin tumors in transgenic TgAC mice in a study using 20 weeks of topical treatment", was based on literature data review (also see a review article, Kraus et al., Benzoyl peroxide: An integrated human safety assessment for carcinogenicity, Regulatory Toxicology and Pharmacology, 21:87-107, 1995). The statements should be included in the label of this NDA. In addition, since the tumors induced in the TgAC mouse study were squamous cell, it is recommended that "squamous cell" be added before the words "skin tumors" in the sentence describing the results of this study.

2.6.6.5 Reproductive and developmental toxicology

No reproductive and developmental toxicology studies have been conducted with Acanya ——— Gel. Since the Sponsor has not established a clinical bridge to the approved drug product BenzaClin Topical Gel to support safety of the proposed drug product. The information on reproductive/developmental toxicity on the labeling of BenzaClin Topical Gel, as shown below, can not be duplicated for this drug product: b(4)

"Studies have not been performed with BenzaClin Topical Gel or benzoyl peroxide to evaluate the effect on fertility. Fertility studies in rats treated orally with up to 300 mg/kg/day of clindamycin (approximately 120 times the amount of clindamycin in the highest recommended adult human dose of 2.5 g BenzaClin Topical Gel, based on mg/m^2) revealed no effects on fertility or mating ability."

"Pregnancy Category C: Animal reproductive/developmental toxicity studies have not been conducted with BenzaClin Topical Gel or benzoyl peroxide. Developmental toxicity studies performed in rats and mice using oral doses of clindamycin up to 600 mg/kg/day (240 and 120 times amount of clindamycin in the highest recommended adult human dose based on mg/m^2, respectively) or subcutaneous doses of clindamycin up to 250 mg/kg/day (100 and 50 times the amount of clindamycin in the highest recommended adult human dose based on mg/m^2, respectively) revealed no evidence of teratogenicity."

However, the sponsor also submitted some published papers to further provide information on the reproductive and developmental toxicity of clindamycin.

a. In the published paper by Gary et al. (The oral toxicity of clindamycin in laboratory animals, Toxicology and Applied Pharmacology 21:516-531, 1972), rats

treated orally with up to 60 mg/kg/day clindamycin hydrochloride or up to 300 mg/kg/day clindamycin palmitate showed no impairment of reproductive performance. In these studies, treatment was started in male rats at 40 days of age and in the females 14 days before breeding, and terminated at the weaning of the F1 generation. Mortality, sex ratios, body weights, and clinical conditions of the offspring were not significantly affected. Reproductive toxicity studies were also conducted in rats and mice with oral clindamycin hydrochloride and clindamycin palmitate. Rats and mice treated orally with up to 200 mg/kg clindamycin hydrochloride and up to 600 mg/kg clindamycin palmitate during days 6 to 15 of gestation did not show any signs of teratogenicity. The information from this published paper may lead to the following statement showing in the labeling of some approved drug products containing clindamycin, "Fertility studies in rats treated orally with up to 300 mg/kg/day of clindamycin revealed no effects on fertility or mating ability" and "Developmental toxicity studies performed in rats and mice using oral doses of clindamycin up to 600 mg/kg/day revealed no evidence of teratogenicity." The same statements should also be included in the label of this NDA.

b. Tanioka et al. [Effects of clindamycin-2-phosphate on the fetuses of experimental animals (I) -- Intraperitoneal administration, The Clinical Report, 7(8):25-37, 1973; Effects of clindamycin-2-phosphate on the fetuses of experimental animals (II) -- Subcutaneous administration, The Clinical Report, 7(8):38-51, 1973] found no fetal mortality in mice injected IP or SC with up to 200 mg/kg/day clindamycin phosphate, respectively, on gestation days 6 to 13. There was a slight delay in fetal ossification by SC administration which was not dose-related. The conclusion was no effect of clindamycin phosphate on the fetus in mice. No fetal mortality or malformations were noted in rats given SC clindamycin phosphate of up to 200 mg/kg on gestation day 9 to 15. Only slight mortality was noted in rats treated by IP administration of up to 200 mg/kg clindamycin phosphate on gestation day 9 to 15.

c. The teratological effects of clindamycin phosphate in mice and rats were also studied by Bollert et al. (Teratogenicity and neonatal toxicity of clindamycin 2-phosphate in laboratory animals, Toxicology and Applied Pharmacology 27:322-329, 1974). Two strains of mice (ICR and CFI) each were injected subcutaneously with 100 and 180 mg/kg/day clindamycin phosphate on gestation days 6 through 15. At these doses there was no detrimental effect on reproduction in either strain nor were there adverse affects on the reproductive parameters evaluated (average live pup weights, average number of live and dead pups per litter, and average number of resorptions per litter). In SD rats injected subcutaneously with 100 and 180 mg/kg/day clindamycin phosphate on gestation days 6 through 15, there was no indication of teratogenic activity in fetuses examined for gross, visceral, and skeletal malformations at these dose levels. Furthermore, the average live pup weight, number of live and dead pups per litter, and average resorptions per litter were similar to those from untreated control rats.

d. However, the sponsor did not submitted any literature to support the following statement shown in the labeling of some approved drug products containing clindamycin, "Developmental toxicity studies performed in rats and mice using subcutaneous doses of clindamycin up to 250 mg/kg/day revealed no evidence of teratogenicity." Based on the

published paper by Tanioka et al. (see above), the subcutaneous doses of clindamycin in this statement should be 200 mg/kg/day.

e. The sponsor also submitted a summary report on the reproductive toxicity of benzoyl peroxide (OECD SIDS 2002). A combined repeated dose and reproduction/developmental toxicity screening test (OECD TG 422) was conducted using SD rats, Four groups of 10 male and 10 female rats were administered oral (gavage) doses of 0, 250, 500 and 1000 mg/kg/day benzoyl peroxide. An additional group of 5 males and 5 females were treated with 4.5 mg/kg/day cyclophosphamide as positive controls. Males were dosed for 29 days starting 14 days before mating through the mating period; females were dosed for 41 to 51 days from 14 days before mating to day 3 of lactation throughout the mating and pregnancy period. "No treatment-related changes in precoital time and rate of copulation, fertility, and gestation were noted in any BPO treated groups. Minimal symptoms, such as vacuolation or hyperplasia, were seen in the 1000 mg/kg group, but this was not considered to have been related to BPO treatment. Adverse effects on reproduction were shown at the highest dose of 1000 mg/kg/day in male rats with the reduction of reproductive organ's weight and slight testes degeneration." "In female rats, no adverse effects were observed during the test period. The NOAEL for reproductive toxicity in male rats was 500 mg/kg/day. No variants were found. High birthrate of runts was seen and body weight gain of pups was significantly decreased (male 9%; female 12.9% of control weight gain) at 1000 mg/kg/day dose. The study concluded that BPO has adverse effects on development of pups with high birthrate or runts at 1000 mg/kg dose level. The NOAEL for developmental toxicity was determined at 500 mg/kg/day." However, information on the reproductive toxicity of benzoyl peroxide from this study is not recommended to be included in the labeling of this NDA, for the following reasons. There were only 10 rats/sex/group in this study and only a summary report was provided. Topically applied benzoyl peroxide is absorbed into the skin and is converted in the skin to benzoic acid. It appears that essentially all of the systemic exposure is to benzoic acid. Benzoic acid is listed as GRAS substance for food and the World Health Organization has established an acceptable daily intake of benzoic acid of 5 mg/kg. So far, no detectable levels of benzoic acid were noted in nonclinical and clinical studies following topical application of [redacted] or Acanya [redacted] Gel. In addition, no statement on the reproductive toxicity of benzoyl peroxide shows in any approved drug product containing benzoyl peroxide.

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2.6.6.6 Local tolerance

The following information on the 2 local tolerance studies was duplicated from the review by Dr. S. R. Joshi within the original submission of IND 41,733 in 1993.

1. Primary Skin Irritation Study in Rabbits with [redacted]

SLS study No. 3242.28 (*91 GLA P007 S007*)

Study Dates: 3/6/92 to 3/9/92.

Materials Tested: Placebo Gel Combination, 5% Benzoyl Peroxide gel, 1% Clindamycin gel, [redacted] gel. b(4)

Animals: New Zealand albino rabbits; 3/sex/test article; 4 groups.

Procedure: A 0.5 g (0.5 ml) of the test material was applied as a single dermal application to both an intact and an abraded test site, approximately 1" x 1" each. Each dose remained unoccluded for an exposure period of 6 hours. Then, each test article was wiped from the skin. Test sites were scored for dermal irritation for up to 72 hours. b(4)

Results: The primary irritation index was 0.29, 0.19, 0.23 and 0.28 for [redacted] clindamycin, benzoyl peroxide and placebo combo, respectively. These scores corresponded to negligible irritant rating.

2. Primary Eve Irritation Study in Rabbits with [redacted]

SLS study No. 3242.27 (*91 GLA P007 S006*)

Study Dates: 3/17/92 to 3/20/92.

Materials Tested: Placebo Gel Combination, 5% Benzoyl Peroxide gel, 1% Clindamycin gel, [redacted] gel.

Animals: New Zealand albino rabbits; 3/ sex/test article; 4 groups.

Procedure: Each of the 12 animals received 0.1 ml of the test article in the right eye; the contralateral left eye served as control. At 30 second post-instillation, both eyes of the 6 rabbits were rinsed (rinsed group), and the eyes of the remaining 6 rabbits were not rinsed (no rinse group). Eyes were examined for up to 72 hours.

Results: The mean ocular irritation scores were: