

**CENTER FOR DRUG EVALUATION AND  
RESEARCH**

*APPLICATION NUMBER:*

**21-794**

**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:	21-794
SERIAL NUMBER:	000
DATE RECEIVED BY CENTER:	07-SEP-04
PRODUCT:	5% Dapsone Gel
INTENDED CLINICAL POPULATION:	Patients with acne vulgaris
SPONSOR:	Atrix Laboratories, Inc.
DOCUMENTS REVIEWED:	All
REVIEW DIVISION:	Division of Dermatologic and Dental Drug Products (HFD-540)
PHARM/TOX REVIEWER:	Norman A. See, Ph.D.
PHARM/TOX SUPERVISOR:	Paul Brown, Ph.D.
DIVISION DIRECTOR:	Jon Wilkin, M.D.
PROJECT MANAGER:	Frank Cross, Jr., M.A., CDR

Date of review submission to Division File System (DFS): 23-May-05

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***EXECUTIVE SUMMARY***

**I. Recommendations**

A. Recommendation on approvability: The product is approvable with respect to nonclinical concerns.

B. Recommendation for nonclinical studies: None.

C. Recommendations on labeling: It is recommended that the "Carcinogenesis" and "Pregnancy" sections of the label be modified as indicated below:

**CARCINOGENESIS, MUTAGENESIS, IMPAIRMENT OF FERTILITY:**





**Pregnancy:**  
**Teratogenic Effects: Pregnancy Category C.**



**II. Summary of nonclinical findings**

A. Brief overview of nonclinical findings: Little toxicity was observed in nonclinical repeat-dose toxicology studies in which the product (or an enriched formulation) was topically applied to skin. No adverse effects were observed in female rats treated daily for six months, although erythrocytic parameters were slightly suppressed in male rats that received dapson gel. No effects were observed in male or female rabbits treated topically for nine months. In rats that were orally dosed with dapson for 90 days, treatment-related findings included cyanosis of the skin, hyperactivity, increased WBC count, decreased RBC count, hemoglobin concentration and hematocrit, increased prothrombin time, splenomegaly, mild splenic "congestion", and

mild pigmentation of the spleen. Potential to induce hemolytic anemia, with splenic involvement, is a known adverse effect of ingested dapsone, but is unlikely to be associated with clinical use of dapsone topical gel due to the low systemic exposure involved. The no-adverse-effect-level (NOAEL) in the 90 day oral rat study was 3 mg/kg/day. The systemic exposures (AUC) achieved at the NOAEL in male and female rats were approximately 15 and 50 times the systemic exposure observed in patients, respectively. Dapsone was negative in an Ames assay (both with and without metabolic activation) and in a micronucleus assay. However, dapsone induced chromosomal aberrations in cultured CHO cells, suggesting that it is a clastogen. Dapsone was evaluated for carcinogenicity in a two-year oral (gavage) rat study and in a Tg.AC mouse study. Both studies were judged by the exec-CAC to be acceptable. No evidence of carcinogenicity was obtained in either study. Dapsone impaired fertility of male rats, as evidenced by a reduction in the fertility index (number of rats pregnant/number of rats mated), reduced sperm motility (percentage of observed sperm that were motile), and reduced numbers of implantations and viable embryos in the females that did become pregnant. However, an oral dose of 2 mg/kg/day had no effect on fertility parameters, and it is unlikely that clinically relevant impairment of fertility would be associated with use of the product. When administered to female rats at a dosage of 75mg/kg/day for 15 days prior to mating and for 17 days thereafter, dapsone reduced the mean number of implantations, increased the mean early resorption rate, and reduced the mean litter size. These effects were probably secondary to maternal toxicity. No effects on the incidence of external, visceral or skeletal malformations or variations were observed. Under the conditions of this study, the NOAEL for dapsone for developmental effects in rats was 12 mg/kg/day. When administered at a dosage of 150 mg/kg/day to rabbits on days 6-18 of gestation, dapsone significantly increased the incidence of early resorptions. Two does at this dosage delivered prematurely and seven does resorbed all fetuses. These effects were probably secondary to maternal toxicity. No effects on the incidence of external, visceral or skeletal malformations or variations were observed. Under the conditions of this study, the NOAEL for dapsone for developmental effects in rabbits was 30 mg/kg/day. Little toxicity was observed in a two-generation study in which F0 females were administered the test articles daily from gestation day 7 through day 27 postpartum at exposures of 3, 12, and 30 mg/kg/day dapsone. The mean number of stillborn pups per litter was slightly, but statistically significantly, higher in high-dose dapsone litters than in control litters. No effects were observed on pup viability, physical development, behavior, learning ability, or reproduction. Dapsone topical gel is not an irritant of skin or eyes, is not phototoxic, and is nonsensitizing.

Please see section 2.6.7 of this review for a tabulated summary of the "safety factors" (AUC ratios at the NOAEL) that were demonstrated in the studies mentioned above.

- B. Pharmacologic activity: Dapsone inhibits growth of certain species of bacteria through inhibition of folic acid synthesis. The mechanism through which dapsone ameliorates acne is unclear, although reducing the bacterial count may reduce the size and quantity of lesions by reducing inflammation.
  
- C. Nonclinical safety issues relevant to clinical use: None, although the label of the product should mention the fact that dapsone induced chromosomal aberrations in cultured CHO cells, suggesting that it is a clastogen, and describe the reproductive toxicology of dapsone, as summarized above.

**APPEARS THIS WAY  
ON ORIGINAL**

## 2.6 PHARMACOLOGY/TOXICOLOGY REVIEW

### 2.6.1 INTRODUCTION AND DRUG HISTORY

**NDA number:** 21-794

**Review number:** 1

**Sequence number/date/type of submission:** N-000/31-AUG-2004

**Information to sponsor:** Yes ( ) No (X)

**Sponsor and/or agent:** Atrix Laboratories, Inc.

**Manufacturer for drug substance:**

**Reviewer name:** Norman A. See, Ph.D.

**Division name:** Division of Dermatologic and Dental Drug Products

**HFD #:** 540

**Review completion date:** 02-MAY-2005

**Drug:**

Trade name: Aczone

Generic name: 5% Dapsone Topical Gel

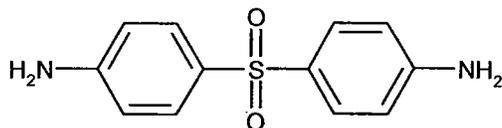
Code name: NA

Chemical name: 4,4'-Sulfonylbisbenzeneamine

CAS registry number: 80-08-0

Molecular formula/molecular weight: C<sub>12</sub>H<sub>12</sub>N<sub>2</sub>O<sub>2</sub>S/248.30

Structure:

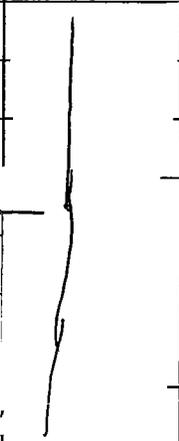


**Relevant INDs/NDAs/DMFs:** NDA 10-039 (Avlosulfon tablets, Wyeth-Ayerst, approved 8/11/55)

**Drug class:** Antimicrobial agent

**Intended clinical population:** Patients with acne vulgaris

**Clinical formulation (topical gel):**

Ingredient	% (w/w)
Dapsone U.S.P.	
DGME*	
Methylparaben U.S.P.	
Carbomer 980	
Sodium Hydroxide U.S.P.	
Purified Water U.S.P.	

\*Also known as diethylene glycol monoethyl ether, ethoxydiglycol, or Transcutol P.

**Route of administration:** Topical to the skin. The proposed use of the product primarily involves application to areas of the head, neck, shoulders, and back that are affected by acne vulgaris. This area comprises approximately 10% of the body surface area. The material would be applied twice daily for an \_\_\_\_\_ resulting in chronic exposure to the product. Approximately 5 to 10 grams of product may be applied per day to a given patient, depending on whether or not application is restricted to the face.

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

**Studies reviewed within this submission:** Note: This product was developed under IND 54,440, which was originally submitted 22-OCT-1997. Some of the studies which support this NDA were reviewed under review formats that were in use at the time the data were originally submitted. Reviews of those studies are included in this NDA in the format under which those studies were originally reviewed and signed off.

**Pharmacokinetic Studies (briefly summarized in PK section):**

1. Pharmacokinetic Profile of Dapsone Following a Single, Dermal Application in Male Sprague-Dawley Rats, study No. ATLS-110.
2. 28-day Dermal Pharmacokinetic Study of Dapsone in Rats, study No. ATLS-181.
3. 28-day Dermal Pharmacokinetic Study of Dapsone in Rabbits, study No. ATLS-182.

**Acute Toxicology:**

1. Acute Oral Toxicity Study in the Rat (FHSA Method), study No. ATLS-89.
2. Acute Dermal Toxicity Study in the Rabbit (FHSA Method), study No. ATLS-91.

**Repeat-Dose Toxicology:**

1. 90-Day Subchronic Oral (Gavage) Toxicity Study in Rats, study No. ATLS-117.
2. A 90-Day Dermal Toxicity Study in Rabbits, study No. ATLS-111.
3. A Six Month Dermal Toxicity Study in Rats, study No. ATLS-114.
4. A 9-Month Dermal Toxicity Study in Rabbits, study No. ATLS-113.

**Genetic Toxicology:**

1. Bacterial Reverse Mutation Assay, study No. ATLS-102.
2. In Vitro Mammalian Chromosomal Aberrations Test, study No. ATLS-101.
3. Mammalian Erythrocyte Micronucleus Test, study No. ATLS-103.
4. Transcutol P Ames Test, study No. TOX 99483.
5. Mammalian Erythrocyte Micronucleus Test with DGME, study No. ATLS-191.
6. Transcutol Measurement of Unscheduled DNA Synthesis in Rat Liver Using an In Vivo/In Vitro Procedure, study No. TOX 96340.

**Carcinogenicity:**

1. 104-Week Carcinogenicity Study of Diethylene Glycol Monoethyl Ether and Dapsone Administered Via Oral Gavage to Sprague-Dawley Rats, study No. ATLS-123.
2. 26-Week Dermal Carcinogenicity Study in Tg.AC Mice, study No. ATLS-163.
3. 12-Month Topical Study to Determine the Influence of Dapsone in a Diethylene Glycol Monoethyl Ether-Based Formulation on Photocarcinogenesis in Hairless Mice, study No. ATLS-122.

**Reproductive Toxicology:**

1. Dapsone and Diethylene Glycol Monoethyl Ether: Oral (Gavage) Fertility and General Reproduction Toxicity Study in Male Rats, study No. ATLS-119.

2. Oral (Gavage) Fertility and General Reproduction Toxicity Study and Recovery Study of Dapsone in Male Rats, study No. ATLS-183.
3. Dapsone and Diethylene Glycol Monoethyl Ether: Combined Oral (Gavage) Fertility and Developmental Toxicity Study in Female Rats, study No. ATLS-120.
4. Dapsone and Diethylene Glycol Monoethyl Ether: Oral (Stomach Tube) Developmental Toxicity Study in Rabbits, study No. 121.
5. Dapsone and Diethylene Glycol Monoethyl Ether: Oral (Gavage) Developmental and Perinatal/Postnatal Reproduction Toxicity Study in Rats, Including a Postnatal Reproduction Toxicity Study in Rats, Including a Postnatal Behavioral/Functional Evaluation, study No. ATLS-137.

**Local Tolerance Studies:**

1. Primary Skin Irritation Study in the Rabbit (FHSA Method), study No. ATLS-94.
2. Ocular Irritation Study in the Rabbit (FHSA Method), study No. ATLS-90.
3. Ocular Irritation Study in the Rabbit (FHSA Method), study No. ATLS-97.

**Special Toxicology Studies:**

1. Topical Phototoxicity Screening Test of Dapsone in a Topical Formulation in Guinea Pigs, study No. ATLS-95a.
2. Topical Phototoxicity Screening Test of Dapsone in a Topical Formulation in Guinea Pigs, study No. ATLS-95b.
3. ISO Sensitization Study in the Guinea Pig (Closed Patch Method), study No. ATLS-98.
4. Delayed Contact Sensitization Study in the Guinea Pig (Repeated Patch Test Method), study No. ATLS-93.

**Studies not reviewed within this submission:** The submission contained a number of photocopies of journal articles that were not specifically summarized in this review because they were judged to add no useful information to the database that was captured in the review. In addition, the following studies were not reviewed because they were judged to be inferior to the studies that were reviewed (listed above), and to add nothing of consequence to the database (they were primarily pilot and preliminary, dose-ranging studies). The nonclinical pharmacokinetic studies, while not reviewed in detail, were summarized in the appropriate sections of this review.

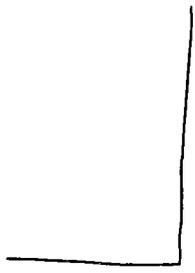
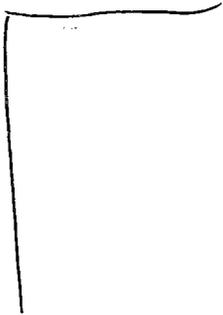
2 Page(s) Withheld

X § 552(b)(4) Trade Secret / Confidential

       § 552(b)(4) Draft Labeling

       § 552(b)(5) Deliberative Process

Withheld Track Number: Pharm/Tox-1



## 2.6.2 PHARMACOLOGY

### 2.6.2.1 Brief summary

Dapsone inhibits growth of certain species of bacteria through inhibition of folic acid synthesis. The mechanism through which dapsone ameliorates acne is unclear, although reducing the bacterial count may reduce the size and quantity of lesions by reducing inflammation.

### 2.6.2.2 Primary pharmacodynamics

Mechanism of action: Dapsone is a structural analog and competitive antagonist of para-aminobenzoic acid, which certain bacterial species use in the synthesis of folic acid. Folic acid is required for growth. However, the method by which dapsone ameliorates acne is unknown. According to the draft label of the product,

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Drug activity related to proposed indication: \_\_\_\_\_

**2.6.2.3 Secondary pharmacodynamics**

None.

**2.6.2.4 Safety pharmacology**

Neurological effects: None known.

Cardiovascular effects: None known.

Pulmonary effects: None known.

Renal effects: None known.

Gastrointestinal effects: None known.

Abuse liability: None known.

Other: None

**2.6.2.5 Pharmacodynamic drug interactions**

None known.

### 2.6.3 PHARMACOLOGY TABULATED SUMMARY

Not available.

### 2.6.4 PHARMACOKINETICS/TOXICOKINETICS

#### 2.6.4.1 Brief summary

Approximately 10% to 25% of a topically applied dose of dapsone was systemically absorbed by rats and rabbits. In humans, less than 1% of a topically applied dose of dapsone is systemically absorbed. Dapsone is rapidly metabolized to N-acetyl dapsone and hydroxylamine dapsone. Dapsone is primarily excreted in the urine.

#### 2.6.4.2 Methods of Analysis

Dapsone and N-acetyl dapsone concentrations in nonclinical samples were analyzed using HPLC and mass spectroscopy (MS). DGME and its metabolites were analyzed using  $\gamma$ -chromatography and MS. The methods used were adequately validated and had acceptable limits of quantitation.

#### 2.6.4.3 Absorption

Studies were conducted in rats and rabbits in which the animals were treated topically with the to-be-marketed formulation (1 mL/kg, or 50 mg dapsone/kg, applied to approximately 10% of the BSA) once daily for 28 consecutive days. The animals wore Elizabethan collars during a four hour daily exposure period to prevent ingestion of the test material. Mean AUC values in rats under those conditions were 32,587 ng·hr/mL in males and 159,928 ng·hr/mL in females, and 14,916 ng·hr/mL and 13,427 ng·hr/mL in male and female rabbits. In a maximum-exposure clinical study in which the to-be-marketed formulation was applied to acne patients twice daily to approximately 20% of the BSA, mean AUC values of 349 ng·hr/mL and 481 ng·hr/mL were observed in males and females, respectively. Therefore, measurable systemic exposure to dapsone occurs following topical application. However, even under maximum exposure conditions of clinical use of the product, the level of exposure to dapsone would be substantially less than that observed in animal studies (see section 2.6.7 of this review for a tabulated comparison of systemic exposure levels at the NOAEL in selected studies).

Following topical dermal application of the to-be-marketed formulation, approximately 10% of the applied dapsone was systemically absorbed by male rats and approximately 25% by female rats. In rabbits, approximately 16% of the applied dapsone was systemically absorbed by both genders. In humans, less than 1% of a topically applied dose of dapsone is systemically absorbed.

#### 2.6.4.4 Distribution

Data not available.

**2.6.4.5 Metabolism**

Dapsone is rapidly metabolized to N-acetyl dapsone and hydroxylamine dapsone. In rabbits following topical dermal dosing of the clinical formulation, plasma  $C_{max}$  and  $AUC_{inf}$  for N-acetyl dapsone were approximately two to three fold higher than those of the parent compound. Plasma  $C_{max}$  and  $AUC_{inf}$  for hydroxylamine dapsone were approximately 5% those of dapsone.

**2.6.4.6 Excretion**

Dapsone is primarily excreted in the urine, with a small percentage being excreted in feces.

**2.6.4.7 Pharmacokinetic drug interactions**

None known.

**2.6.4.8 Other Pharmacokinetic Studies**

Not applicable.

**2.6.4.9 Discussion and Conclusions**

Approximately 10% to 25% of a topically applied dose of dapsone was systemically absorbed by rats and rabbits. In humans, less than 1% of a topically applied dose of dapsone is systemically absorbed. Dapsone is rapidly metabolized to N-acetyl dapsone and hydroxylamine dapsone. Dapsone is primarily excreted in the urine.

**2.6.4.10 Tables and figures to include comparative TK summary****Interspecies Comparison of Pharmacokinetic Parameters**

Species (Route)	No. of Doses*	Dose (mg/kg/dose)	$C_{max}$ of Males (ng/mL)	$C_{max}$ of Females (ng/mL)	AUC of Males (ng·hr/mL)	AUC of Females (ng·hr/mL)
Rat (Topical)	28	50	2613	10909	32587	159928
Rabbit (Topical)	28	50	1469	1864	14916	13427
Human (Topical)	28	1.37	17.1	22.3	349	481
Human (Oral)	1	1.67	1375	NA	52641	NA

\*The rat and rabbit data were obtained in studies involving once daily dosing for 28 consecutive days. The rats and rabbits in these studies wore Elizabethan collars during a four hour exposure period each day, followed by washing of the application site. The clinical topical data were obtained from a study in which patients were dosed twice daily (the proposed clinical regimen) for 14 days, and the to-be-marketed formulation was applied to approximately 20% of the body surface area. The human oral data are from a study in which subjects ingested a single 100 mg tablet of dapson.

See section 2.6.7 of this review for a tabulated comparison of systemic exposure levels at the NOAEL in selected studies.

## 2.6.5 PHARMACOKINETICS TABULATED SUMMARY

See above.

## 2.6.6 TOXICOLOGY

### 2.6.6.1 Overall toxicology summary

General toxicology: Substantial toxicity was not observed in chronic toxicology studies in which dapson topical gel was dermally applied. No adverse effects were observed in female rats treated daily for six months, although the mean RBC, HGB, and HCT values of male dapson-treated animals were slightly, but significantly reduced, and the mean weight of the spleen was significantly increased in male rats that received dapson gel. No effects were observed in male or female rabbits treated topically for nine months.

In rats that were orally dosed for 90 days, treatment-related findings observed at 30mg/kg/day included cyanosis of the skin, hyperactivity, increased WBC count, decreased RBC count, hemoglobin concentration and hematocrit, increased prothrombin time, splenomegaly, mild splenic "congestion", and mild pigmentation of the spleen. These effects and more were observed at 100mg/kg/day. 3mg/kg/day was an apparent no-adverse-effect-level (NOAEL) in that study. 180mg/kg/day DGME was also a NOAEL in that study.

Genetic toxicology: Dapson was negative in an Ames assay (both with and without metabolic activation) and in a micronucleus assay. However, dapson induced chromosomal aberrations in cultured CHO cells, suggesting that it is a clastogen.

DGME was negative in an Ames assay, an unscheduled DNA synthesis assay, and in a micronucleus assay.

Carcinogenicity: Dapson was evaluated for carcinogenicity in a two-year oral (gavage) rat study and in a Tg.AC mouse study. Both studies were judged by the exec-CAC to be acceptable. No evidence of carcinogenicity was obtained in either study.

Reproductive toxicology: Dapsone impaired fertility of male rats, as evidenced by a reduction in the fertility index (number of rats pregnant/number of rats mated), reduced sperm motility (percentage of observed sperm that were motile), and reduced numbers of implantations and viable embryos in the females that did become pregnant. Statistically significant reductions in percentage of motile sperm were observed at exposures of 3 mg/kg/day and above. 2 mg/kg/day was an apparent NOAEL for effects on male fertility. DGME had no effects on fertility.

When administered to female rats at a dosage of 75mg/kg/day for 15 days prior to mating and for 17 days thereafter, dapsone reduced the mean number of implantations, increased the mean early resorption rate, and reduced the mean litter size. These effects were probably secondary to maternal toxicity. No effects on the incidence of external, visceral or skeletal malformations or variations were observed. Under the conditions of this study, the NOAELs for dapsone and DGME were 12 mg/kg/day and 180 mg/kg/day, respectively. DGME did not induce toxicity under the conditions of this study.

When administered at a dosage of 150 mg/kg/day to rabbits on days 6-18 of gestation, dapsone significantly increased the incidence of early resorptions. Two does at this dosage delivered prematurely and seven does resorbed all fetuses. These effects were probably secondary to maternal toxicity. No effects on the incidence of external, visceral or skeletal malformations or variations were observed. Under the conditions of this study, the NOAELs for dapsone and DGME were 30 mg/kg/day and 180 mg/kg/day, respectively. DGME did not induce toxicity under the conditions of this study.

Little toxicity was observed in a two-generation study in which F0 females were administered the test articles daily from gestation day 7 through day 27 postpartum at exposures of 3, 12, and 30 mg/kg/day dapsone or 180 mg/kg/day DGME. The mean number of stillborn pups per litter was slightly, but statistically significantly, higher in high-dose dapsone litters than in control litters. No effects were observed on pup viability, physical development, behavior, learning ability, or reproduction.

Special toxicology: Dapsone topical gel is not an irritant of skin or eyes, is not phototoxic, and is nonsensitizing.

#### **2.6.6.2 Single-dose toxicity**

**2.6.6.2.1. Acute oral toxicity study in the rat**, study No. TA004-900, sponsor study No. ATLS-89, in-life 6/97-7/97, study report dated 7/15/97, conducted by ) \_\_\_\_\_

\_\_\_\_\_ in compliance with Good Laboratory Practice regulations (21 CFR 58).

Ten CrI:CD(SD)BR rats (5 per gender) were used. Each rat received 5g/kg of the test article (1% dapsone gel, formula #05/44-1, lot #020-97) by gavage. The rats were monitored for 14 days for signs of illness or mortality. Body weights were recorded at the time of dosing and after 14 days.

**Results**: No effects on body weight, survival, clinical signs, or gross necropsy were observed.

**Conclusions:** Under the conditions of this study, the test article (1% dapsone gel) did not appear to induce toxicity.

**2.6.6.2.2 Acute dermal toxicity study in the rabbit**, study No. TA003-800, sponsor study No. ATLS-91, in-life 7/97-8/97, study report dated 8/8/97, conducted by \_\_\_\_\_, in compliance with

Good Laboratory Practice regulations (21 CFR 58).

Ten New Zealand white rabbits (5 per gender) were used. The back of each animal was shaved. Half the animals received epidermal abrasions on the shaved area. The test article (1% dapsone gel, formula #05/44-1, lot #020-97) was applied to the back of each animal at a dose of 2g/kg and covered with polyethylene plastic. The rabbits were fitted with collars and the test article was left in place for 24 hours. The test sites were then wiped clean and the rabbits were monitored for 14 days for signs of illness, mortality, and dermal reactions (erythema and edema) at the site of application. Body weights were recorded at the time of dosing and after 14 days.

**Results:** Very slight (barely perceptible) erythema was observed for the first 7 to 12 days after treatment; this may have been due to shaving, occlusion, taping, etc. No edema was observed. No effects on body weight, survival, clinical signs, or gross necropsy were observed.

**Conclusions:** Under the conditions of this study, the test article did not appear to induce toxicity.

### 2.6.6.3 Repeat-dose toxicity

#### 2.6.6.3.1 Study title: 90-day subchronic oral (gavage) toxicity study in rats

**Key study findings:** Treatment-related findings observed at 30mg/kg/day included cyanosis of the skin (dapsone is known to induce methemoglobinemia, and the cyanosis may have been secondary to this), hyperactivity, increased WBC count, decreased RBC count, hemoglobin concentration and hematocrit, increased prothrombin time, splenomegaly (especially in males), mild splenic "congestion", and mild pigmentation of the spleen. These effects and more were observed at 100mg/kg/day. No frank toxicity was observed at 3mg/kg/day dapsone, although "minimal" brown pigmentation of the spleen was observed. I consider 3mg/kg/day to have been a NOAEL in this study. 180mg/kg/day DGME was also a NOAEL.

**Study No:** 128-001

**Amendment #, Vol #, and page #:** 032, 1, B1

**Conducting laboratory and location:** \_\_\_\_\_

**Date study initiated:** 22-MAR-2000

**Animal phase initiation:** 23-MAR-2000

**Date of final sign-off by study director:** 18-OCT-2001

**GLP compliance:** Yes

**QA- Report** Yes (X) No ( )

**Methods:**

## Dosing:

- species/strain: Rat/Crl:CD(SD) albino
- #/sex/group or time point: 10; additional 10 per sex per group for toxicokinetic purposes only
- age: Six weeks at initiation
- weight: Males 123-175g, females 131-157g
- satellite groups used for toxicokinetics or recovery: Yes
- summary of study design:

Group Number	Dosages, Test Article 1/Test Article 2 (mg/kg/day)*	No. of Rats (main study/TK study)	
		Male	Female
1	0/0 (vehicle)	10/0	10/0
2	0/180	10/10	10/10
3	3/0	10/10	10/10
4	30/0	10/10	10/10
5	100/0	10/10	10/10

\*Test article 1 is dapson; test article 2 is diethylene glycol monoethyl ether (DGME; an excipient in the product)

- treatment: Main study animals, 90 consecutive days; TK animals, approximately 114 consecutive days
- route, form, volume: Oral (gavage), solution/suspension, 10mL/kg
- drug, lot#, and % purity: Dapson, 01110C, assumed  $\frac{100}{100}$  % purity; DGME, 11189A, assumed  $\frac{100}{100}$  % purity
- Formulation/vehicle: Suspended in 0.5% carboxymethylcellulose

## Observations:

- Survival: Yes
- Clinical signs: Yes (twice daily)
- Body weights: Yes (weekly)
- Food consumption: Yes (weekly)
- Ophthalmoscopy: Yes
- EKG: No
- Hematology: Yes (on day 85)
- Clinical chemistry: Yes (on day 85)
- Urinalysis: No
- Organ weights: Yes
- Gross pathology: Yes
- Organs weighed: Adrenals, brain, kidneys, liver, ovaries, spleen, testes, thymus

- Histopathology: Yes, of main study animals in groups 1, 2, and 5, and gross lesions, liver, lungs, kidneys, target organs (spleen), and tumors from animals in groups 3 and 4
- List of tissues histologically examined: Standard list
- Toxicokinetics: Yes; samples obtained from three animals per group per time point on days 1 and 90 at 0 (immediately prior to treatment), 0.5, 1, 2, 4, 8, and 24 hours post-dose. The  $C_{max}$ ,  $T_{max}$ , time of last measurable concentration ( $T_{last}$ ), and  $AUC_{0-24}$  were determined.

**Results:**

- Survival: No remarkable unscheduled deaths.
- Clinical signs: Skin discoloration (cyanosis) of mouth, nose, limbs, ears, and body in 3/10 males (but no females) at 3mg/kg/day and in both males and females at 30mg/kg/day or above.  
Hyperactivity in both males and females at 30mg/kg/day or above.
- Body weights: Significantly reduced in males at 100mg/kg/day (day 90 mean weights of group 1 and group 5 males were  $561 \pm 74g$  and  $457 \pm 25g$ , respectively). The body weight gains were reduced for all male treatment groups relative to controls, although the differences were not significant. Nonsignificant trend toward reduced mean body weight in females at 100mg/kg/day (day 90 mean weights of group 1 and group 5 females were  $275 \pm 18g$  and  $264 \pm 18g$ , respectively).
- Food consumption: Significantly reduced in males at 100mg/kg/day.
- Ophthalmology: No remarkable observations.
- Hematology: Significant differences were observed in a number of hematological parameters in both males and females at 30mg/kg/day or above, including increased WBC count (lymphocytes and segmented neutrophils), decreased RBC count, decreased hemoglobin, reduced hematocrit (males exhibited a nonsignificant trend only), increased mean corpuscular volume and mean corpuscular hemoglobin (males only), and increased prothrombin time.
- Clinical chemistry: Significant differences were observed in a number of clinical chemistry parameters in both males and females at 100mg/kg/day, including, in males, increased levels/activities of albumin, bilirubin, BUN, alanine aminotransferase, gammaglutamyltransferase, and potassium, and decreased levels of triglycerides and chloride, while females exhibited increased BUN, alanine aminotransferase, alkaline phosphatase, and calcium, and reduced chloride. At 30mg/kg/day, males exhibited significantly reduced cholesterol, elevated bilirubin, and elevated potassium, while females exhibited significantly elevated BUN and calcium.
- Urinalysis: Not performed.
- Absolute Organ Weights: Significantly increased mean spleen weight at 30mg/kg/day and above (males, 200%-300% increase; females 20%-40% increase). In females, increased mean liver weight at 30mg/kg/day and above (approximately 25% increase).
- Gross pathology: In males at 30mg/kg/day and above, nearly all exhibited an

enlarged spleen. "Small thymus" was observed in 3/10 males at 30mg/kg/day and 9/10 males at 100mg/kg/day. In females at 100mg/kg/day, 7/10 exhibited uterine enlargement.

- Histopathology: Treatment-related findings were limited to the spleen. Mild splenic congestion and minimal extramedullary hematopoiesis were observed in male animals at 30mg/kg/day and above. Minimal brown pigmentation of the spleen was observed in both males and females at 3mg/kg/day, while animals at 30mg/kg/day and above exhibited mild (more severe than minimal) pigmentation of the spleen.

- Toxicokinetics:

Dosage	Males, Day 90					Females, Day 90				
	AUC <sub>0-24</sub> ( $\mu\text{g}\cdot\text{hr}/\text{mL}$ )	AUC <sub>0-inf</sub> ( $\mu\text{g}\cdot\text{hr}/\text{mL}$ )	C <sub>max</sub> ( $\mu\text{g}/\text{mL}$ )	T <sub>max</sub> (hr)	T <sub>1/2</sub> (hr)	AUC <sub>0-24</sub> ( $\mu\text{g}\cdot\text{hr}/\text{mL}$ )	AUC <sub>0-inf</sub> ( $\mu\text{g}\cdot\text{hr}/\text{mL}$ )	C <sub>max</sub> ( $\mu\text{g}/\text{mL}$ )	T <sub>max</sub> (hr)	T <sub>1/2</sub> (hr)
3 mg/kg/day (Group 3)	6.25	6.40	0.84	0.5	4.5	20.8	24.8	2.01	0.5	9.2
30 mg/kg/day (Group 4)	132	155	8.78	2.0	9.6	252	NC	13.9	4.0	NC
100 mg/kg/day (Group 5)	431	NC	22.8	8.0	NC	529	1084	39.1	1.0	24.8

NC indicates "not calculated", as terminal phase was not adequately defined.

#### 2.6.6.3.2 Study Title: A 90-day dermal toxicity study in rabbits

**Key study findings:** No toxicity was observed under the conditions of this study, which included application of the clinical formulation, as well as a formulation that contained twice the concentration proposed for clinical use. Dapsone was absorbed into the blood in proportion to the amount (concentration) of dapsone applied.

**Study No:** 0470LA56.001

**Amendment #, Vol #, and page #:** 024, 2/3, D1

**Conducting laboratory and location:** \_\_\_\_\_

**Date protocol initially signed by study director:** 7-MAY-1999

**Date treatment initiated:** 12-MAY-1999

**Date of final sign-off by study director:** 8-MAR-2000

**GLP compliance:** Yes

**QA-Report: Yes****Methods:****Dosing:**

- species/strain: Rabbit/New Zealand White - HM(NZW)fBR
- #/sex/group or time point: 10
- age: 12 weeks at initiation
- weight: Males 2.0-2.3kg, females 1.8-2.4kg
- satellite groups used for toxicokinetics or recovery: No
- Summary of study design:

Group (Dapsone Dose)	Test Article	No. of Rabbits	
		Male	Female
1. Vehicle (0 mg/kg/day)	40% DGME gel	10	10
2. Low Dose (10mg/kg/day)	1% Dapsone, 10% DGME gel	10	10
3. Mid-Dose (50mg/kg/day)	5% Dapsone, 25% DGME gel	10	10
4. High Dose (100mg/kg/day)	10% Dapsone, 40% DGME gel	10	10

Treatment consisted of application of 1ml/kg of the assigned material over a shaved area consisting of 10% to 20% of the body surface area which was occluded for four hours with a gauze pad, a rubber dam, and an elastic bandage. At the end of the daily exposure period the occlusion materials were removed and the application site was gently cleansed with a gauze soaked in warm water and then dried. This procedure was repeated for 90 consecutive days.

- route, form, volume: Topical dermal (interscapular/dorsal thoracic), gel, 1ml/kg

Formulation/vehicle: The test materials were apparently the clinical formulations, varying only in the concentration of dapsone and DGME.

**Observations:**

- Survival: Yes
- Clinical signs: Yes (twice daily)
- Body weights: Yes (weekly)
- Food consumption: Yes (daily)
- Ophthalmoscopy: No
- EKG: No
- Hematology: Yes (prior to first treatment and on day 91)
- Clinical chemistry: Yes (prior to first treatment and on day 91)
- Urinalysis: No
- Organ weights: Yes
- Gross pathology: Yes
- Organs weighed: Adrenals, brain, kidneys, liver, ovaries, spleen, testes

- Histopathology: Yes, of control group and the high dose group plus treatment sites (skin) and gross lesions from the low and mid-dose groups and tissues from all unscheduled deaths
- List of tissues histologically examined: Standard list
- Toxicokinetics: Yes (males only); samples obtained from three male animals per group per time point on days 1 and 90 at 0 (immediately prior to treatment), 1, 2, 4, 6, and 24 hours post-dose.

**Results:**

- Survival: No remarkable unscheduled deaths.
- Clinical signs: No remarkable observations.
- Body weights: No remarkable observations.
- Food consumption: No remarkable observations.
- Hematology: No remarkable observations.
- Clinical chemistry: No remarkable observations.
- Urinalysis: Not performed.
- Organ Weights: No remarkable observations.
- Gross pathology: No remarkable observations.
- Histopathology: No remarkable observations.
- Toxicokinetics: (Data obtained in males only)

Test Material	Day 1				Day 90			
	AUC <sub>0-24</sub> (ng•hr/ mL)	C <sub>max</sub> (ng/mL)	T <sub>max</sub> (hr)	T <sub>1/2</sub> * (hr)	AUC <sub>0-24</sub> (ng•hr/ mL)	C <sub>max</sub> (ng/mL)	T <sub>max</sub> (hr)	T <sub>1/2</sub> * (hr)
1% Dapsone/10 % DGME	65.9	7.6	2	24	226	15	6	17
5% Dapsone/25 % DGME	324	28	6	5.4	1060	59	6	9.5
10% Dapsone/40 % DGME	606	33	6	17	1590	120	6	1.4

\*Calculated from T<sub>max</sub> to 24 hour value.

Key Study Findings: No toxicity observed. Dapsone was absorbed into the blood in proportion to the amount (concentration) of dapsone applied.

### 2.6.6.3.3 Study title: A six month dermal toxicity study in rats

**Key study findings:** Little toxicity was observed under the conditions of this study, which included application of the clinical formulation, as well as a formulation that contained twice the concentration proposed for clinical use. The mean RBC, HGB, and

HCT values of both low and high-dose male dapsone-treated animals were slightly, but significantly reduced. The mean weight of the spleen (both absolute and relative to body weight) was significantly increased in male rats of both dapsone treatment groups. The Dapsone was absorbed into the blood in proportion to the amount (concentration) of dapsone applied. A true NOAEL was not observed in this study (in view of the slight effects on erythrocytic parameters and spleen weight), but as a practical matter one could consider the highest exposure studied (10% dapsone/40% DGME gel) to have been essentially a NOAEL. I will use toxicokinetic data from that group for the purpose of calculating the "no-effect" AUC ratio.

**Study No:** ATLS-114

**Amendment #, Vol #, and page #:** Mod 4, vol 28

**Conducting laboratory and location:** \_\_\_\_\_

**Date protocol initially signed by study director:** 12-NOV-1999

**Date treatment initiated:** 30-NOV-1999

**Date of final sign-off by study director:** 10-OCT-2002

**GLP compliance:** Yes

**QA-Report:** Yes

**Methods:**

**Dosing:**

- species/strain: Rat/Sprague-Dawley-Hsd:SD(CD)
- #/sex/group or time point: 10
- age: 7 weeks at initiation
- weight: Males 248-301 g, females 139-201 g
- satellite groups used for toxicokinetics or recovery: Yes, 6 animals/sex at both 5% dapsone/25% DGME gel and 10% dapsone/40% DGME gel
- Summary of study design:

Group (Dapsone Dose)	Test Article	No. of Rats	
		Male	Female
1. Negative Control (0 mg/kg/day)	Deionized Water	10	10
2. Vehicle Control (0mg/kg/day)	40% DGME gel (vehicle for 10% dapsone test material)	10	10
3. Low-Dose (50mg/kg/day)	5% Dapsone, 25% DGME gel	10 (6)*	10 (6)
4. High Dose (100mg/kg/day)	10% Dapsone, 40% DGME gel	10 (6)	10 (6)

\*Numbers in parenthesis indicate number of TK animals.

Treatment consisted of application of 1 ml/kg of the assigned material over a shaved area consisting of 10% to 15% of the body surface area which was occluded for four hours with a gauze pad, a rubber dam, and an elastic bandage.

At the end of the daily exposure period (consisting of four hours) the occlusion materials were removed and the application site was gently cleansed with a gauze soaked in warm water and then dried. This procedure was repeated for approximately 180 consecutive days.

- route, form, volume: Topical dermal (interscapular/dorsal thoracic), gel, 1 ml/kg

Formulation/vehicle: The test materials were apparently the clinical formulation, varying only in the concentration of dapsone and DGME.

**Observations:**

- Survival: Yes (twice daily)
- Clinical signs: Yes (twice daily), pre- and post-treatment
- Body weights: Yes (weekly)
- Food consumption: Yes (weekly)
- Ophthalmoscopy: Yes
- EKG: No
- Hematology: Yes (on day 183)
- Clinical chemistry: Yes (on day 183)
- Urinalysis: Yes
- Organ weights: Yes
- Gross pathology: Yes
- Organs weighed: Adrenals, brain, kidneys, liver, ovaries, spleen, testes, thyroid/parathyroid
- Histopathology: Yes, of groups 1 (water control), 2 (vehicle control), and 4 (high dose group) plus treatment sites (skin) and gross lesions from group 3 (low dose group) animals. Mesenteric fat samples from group 3 females were also examined.
- List of tissues histologically examined: Standard list
- Toxicokinetics: Yes, from group 5 and 6 TK satellite animals (see table above). Blood samples obtained from three animals per sex per group per time point on day 1 of dosing at 0 (immediately prior to treatment), 1, 2, 4, 6, and 24 hours post-dose. Samples were obtained on day 183 at the same time points, but the numbers of animals sampled were slightly reduced due to mortality.

**Results:**

- Survival: No remarkable unscheduled deaths in the "toxicologic" portion of the study, although one male and one female in the 10% dapsone/40% DGME group in the toxicokinetic portion of the study died prematurely. The cause of those deaths is not discussed in the study report.
- Clinical signs: No remarkable observations, including no irritation, erythema, or edema at the application site.
- Body weights: No remarkable observations.
- Food consumption: No remarkable observations.
- Hematology: The mean RBC, HGB, and HCT values of both low and high-dose male dapsone-treated animals were slightly, but significantly reduced:

Hematology Parameters, Male Rats:

Group	Mean RBC Count ( $1 \times 10^6/\text{mL}$ )	Hemoglobin Conc. (g/dL)	Hematocrit (%)
1 (Water)	8.38±0.332	15.3±0.43	48.0±1.76
2 (Vehicle)	8.30±0.365	15.0±0.55	46.6±2.21
3 (5% Dapsone gel)	7.32±0.303**	14.0±0.54**	45.1±1.63**
4 (10% Dapsone gel)	7.54±0.231**	14.0±0.28**	45.1±1.58**

\*\*p&lt;0.01

No remarkable changes in the hematology of female rats were observed.

- Clinical chemistry: No remarkable observations.
- Urinalysis: No remarkable observations.
- Organ Weights: Mean weight of the spleen (both absolute and relative to body weight) were significantly increased in male rats of both dapsone treatment groups:

Mean Spleen Weight, Males:

Group	Mean Absolute Spleen Weight (grams)
1 (Water)	0.91±0.185
2 (Vehicle)	0.83±0.070
3 (5% Dapsone gel)	1.28±0.193**
4 (10% Dapsone gel)	1.16±0.194**

\*\*p&lt;0.01

No other remarkable organ-weight changes in males, or in females, were observed.

- Gross pathology: No remarkable observations.
- Histopathology: No remarkable observations.
- Toxicokinetics:

Test Material	Day 1				Day 182			
	AUC <sub>0-24</sub> (ng•hr/ mL)	C <sub>max</sub> (ng/mL)	T <sub>max</sub> (hr)	T <sub>1/2</sub> * (hr)	AUC <sub>0-24</sub> (ng•hr/ mL)	C <sub>max</sub> (ng/mL)	T <sub>max</sub> (hr)	T <sub>1/2</sub> * (hr)
Males: 5% Dapsone/25 % DGME	478	25.3	6	NA	1837	117.8	1	NA
Males: 10% Dapsone/40 % DGME	1064	53.4	6	NA	11294	653	24	NA

Females: 5% Dapsone/25 % DGME	3475	187.7	6	NA	6676	366.3	1	NA
Females: 10% Dapsone/40 % DGME	6065	320.3	24	NA	11102	841.3	0	NA

**2.6.6.3.4 Study title:** A 9-month dermal toxicity study in rabbits, study No. ATLS-113.

**Key study findings:** No toxicity was observed under the conditions of this study, which included application of the clinical formulation, as well as a formulation that contained twice the concentration proposed for clinical use. Dapsone was absorbed into the blood in proportion to the amount (concentration) of dapsone applied. 10% dapsone/40% DGME gel was a NOAEL under the conditions of this study.

**Study No:** ATLS-113

**Amendment #, Vol #, and page #:** Mod 4, vol 35

**Conducting laboratory and location:** \_\_\_\_\_

**Date protocol initially signed by study director:** 12-NOV-1999

**Date treatment initiated:** 02-DEC-1999

**Date of final sign-off by study director:** 11-OCT-2002

**GLP compliance:** Yes

**QA-Report:** Yes

**Methods:**

**Dosing:**

- species/strain: Rabbit/New Zealand White - HM(NZW)fBR
- #/sex/group or time point: 8
- age: 15 weeks at initiation
- weight: Males 2.4-2.9 kg, females 2.5-3.0 kg
- satellite groups used for toxicokinetics or recovery: No
- Summary of study design:

Group (Dapsone Dose)	Test Article	No. of Rabbits	
		Male	Female
1. Negative Control (0 mg/kg/day)	Deionized Water	8	8
2. Vehicle Control (0mg/kg/day)	40% DGME gel (vehicle for 10% dapsone gel)	8	8

3. Low-Dose (50mg/kg/day)	5% Dapsone, 25% EDG	8	8
4. High Dose (100mg/kg/day)	10% Dapsone, 40% EDG	8	8

Treatment consisted of application of 1 ml/kg of the assigned material over a shaved area consisting of 10% to 15% of the body surface area which was occluded for four hours with a gauze pad, a rubber dam, and an elastic bandage. At the end of the daily exposure period the occlusion materials were removed and the application site was gently cleansed with a gauze soaked in warm water and then dried. This procedure was repeated for 273 consecutive days.

- route, form, volume: Topical dermal (interscapular/dorsal thoracic), gel, 1 ml/kg

Formulation/vehicle: The test materials were apparently the clinical formulations, varying only in the concentration of dapsone and DGME.

**Observations:**

- Survival: Yes (twice daily)
- Clinical signs: Yes (twice daily, pre and post-treatment)
- Body weights: Yes (weekly)
- Food consumption: Yes (daily)
- Ophthalmoscopy: Yes, prior to first treatment and during week 39
- EKG: No
- Hematology: Yes (prior to first treatment and on day 274)
- Clinical chemistry: Yes (prior to first treatment and on day 274)
- Urinalysis: No
- Organ weights: Yes
- Gross pathology: Yes
- Organs weighed: Adrenals, brain, kidneys, liver, ovaries, spleen, testes, thyroids/parathyroids
- Histopathology: Yes, of groups 1 (water control), 2 (vehicle control), and 4 (high dose group) plus treatment sites (skin) and gross lesions from group 3 (low dose group) animals.
- List of tissues histologically examined: Standard list
  
- Toxicokinetics: Yes; samples obtained from two or three animals of each gender per group per time point on days 1 and 273 at 0 (immediately prior to treatment), 1, 2, 4, 6, and 24 hours post-dose.

**Results:**

- Survival: No remarkable unscheduled deaths.
- Clinical signs: No remarkable observations, including no irritation, erythema, or edema at the application site.
- Body weights: No remarkable observations.
- Food consumption: No remarkable observations.
- Ophthalmology: No remarkable observations.

- Hematology: No remarkable observations.
- Clinical chemistry: No remarkable observations.
- Urinalysis: Not performed.
- Organ Weights: No remarkable observations.
- Gross pathology: No remarkable observations.
- Histopathology: No remarkable observations.
- Toxicokinetics:

Test Material	Day 1				Day 273			
	AUC <sub>0-24</sub> (ng•hr/ mL)	C <sub>max</sub> (ng/mL)	T <sub>max</sub> (hr)	T <sub>1/2</sub> * (hr)	AUC <sub>0-24</sub> (ng•hr/ mL)	C <sub>max</sub> (ng/mL)	T <sub>max</sub> (hr)	T <sub>1/2</sub> * (hr)
Males, 5% Dapsone/ 25% DGME	383	34	6	NA	3240	262	6	NA
Males, 10% Dapsone/ 40% DGME	1746	163	6	NA	7671	549	6	NA
Females, 5% Dapsone/ 25% DGME	722	60	6	NA	2293	155	6	NA
Females, 10% Dapsone/ 40% DGME	1648	145	6	NA	5470	431	6	NA

#### 2.6.6.4 Genetic toxicology

**2.6.6.4.1 Study title:** Bacterial reverse mutation assay, ~~Study No.~~ Study No. G98AY64.502, sponsor's ID No. ATLS-102, report dated 4/27/99, conducted by ~~\_\_\_\_\_~~ in compliance with Good Laboratory Practice regulations (21 CFR 58).

*S. typhimurium* strains TA 98, TA 100, TA 1535, TA 1537, and *E. coli* strain WP2 *uvrA* were plated with dapsone; the amount of test material per plate ranged from 75 to 5000 µg. Assays were conducted with and without metabolic activation (S9). Appropriate positive control compounds were used.

**Results.** No significant increase in the reverse mutation rate was observed at any concentration of test material, in either the presence or absence of S9. Appropriate responses were induced by the positive control substances.

**Conclusions.** This study provided no evidence that the test material was mutagenic.

**2.6.6.4.2 Study title:** *In vitro* mammalian chromosomal aberration test, \_\_\_\_\_ Study No. G98AY64.331, sponsor's ID No. ATLS-101, report dated 4/5/99, conducted by \_\_\_\_\_ in compliance with Good Laboratory Practice regulations (21 CFR 58).

Dapsone was assayed for the ability to induce chromosomal aberrations in cultured CHO-K<sub>1</sub> cells, both in the presence and in the absence of metabolic activation (S9). The cells were exposed to dapsone in concentrations ranging up to 1500 µg/ml; assays were conducted with and without S9 according to standard procedures. Assays involving both 4 hour and 20 hour incubations were conducted. DMSO (the solvent) was used as a negative control; cyclophosphamide was used as a positive control in experiments with S9, and mytomycin C was used as a positive control in experiments without S9. All cultures were treated with colchicine prior to harvest. Prepared slides were examined for chromosomal aberrations.

**Results.** Exposure to dapsone significantly increased the frequency of occurrence of chromosomal aberrations in the absence of metabolic activation (but not in the presence of S9 under the experimental conditions). Dapsone increased numerical aberrations at 750 µg/mL and structural aberrations at 1500 µg/mL during a 4 hour incubation, and induced structural aberrations at 750 µg/mL during a 20 hour exposure. Appropriate responses were obtained with the control materials.

**Conclusions.** Dapsone induced chromosomal aberrations in cultured CHO cells, suggesting that it is a clastogen.

**2.6.6.4.3 Study title:** Mammalian erythrocyte micronucleus test, \_\_\_\_\_ Study No. G98AY64.123, sponsor's ID No. ATLS-103, report dated 2/2/99, conducted by \_\_\_\_\_ in compliance with Good Laboratory Practice regulations (21 CFR 58).

Dapsone was assessed for effect on the incidence of micronucleated polychromatic erythrocytes in ICR mice. The animals each received a single IP injection of either 160, 320, or 640mg/kg dapsone, water (negative control), or cyclophosphamide (positive control, 50mg/kg). Bone marrow smears were obtained from negative control and dapsone-treated mice (5/sex/dose) at 24 and 48 hours post-dosing; positive controls (5/sex) were sacrificed at 24 hours only. The smears were processed and examined for the number of micronucleated polychromatic cells per 2000 polychromatic erythrocytes examined; the ratios of polychromatic to normochromatic erythrocytes were recorded.

**Results.** No statistically significant differences were observed between the test substance and the negative control. A significant increase in the occurrence of micronucleated cells relative to the number of polychromatic erythrocytes was observed in smears from positive control animals. Ataxia and convulsions were observed at all dosages of dapsone studied, and three animals died at 640mg/kg.

**Conclusions.** These data provide no evidence that the test substance is clastogenic.

**2.6.6.4.4 Study title:** Transcutol P Ames Test

**Key findings:** Transcutol (DGME) was not mutagenic in an Ames assay.

**Study no:** TOX 99483

**Study type** (if not reflected in title): Ames test

**Volume #, and page #:** Mod 4, vol 40

**Conducting laboratory and location:** \_\_\_\_\_

**Date of study initiation:** 18-NOV-1998

**GLP compliance:** Yes

**QA reports:** yes (X) no ( )

**Drug, lot #, radiolabel, and % purity:** Transcutol P (Diethylene glycol monoethyl ether; DGME), lot 9833703,  %

**Formulation/vehicle:** DGME dissolved in water at concentrations up to 50 mg/mL

**Methods:**

Strains/species/cell line: Salmonella strains TA98, TA100, TA1535, TA1537; TA102

Dose selection criteria: Compound was not cytotoxic; therefore, 5 mg per plate used as maximum exposure level per ICH S2A document

Basis of dose selection: ICH S2A

Range finding studies: Yes, at exposures up to 5 mg/plate

Test agent stability: NA

Metabolic activation system: Rat liver S9; induced with mixture of phenobarbital and methylcholanthrene

Controls:

Negative control: vehicle

Positive controls: 2-aminoanthracene (all strains, +S9); 2-nitrofluorene (TA98, -S9); sodium azide (TA100 & TA1535, -S9); 9-aminoacridine (TA1537, -S9); t-butyl hydroperoxide (TA102, -S9)

Comments: Controls adequate

Exposure conditions:

Incubation and sampling times: 48 to 72 hrs

Doses used in definitive study: 15 to 5000 µg per plate

Study design: Plate method

Analysis:

No. of replicates: 2

Counting method: Automated counter or by hand

Criteria for positive results: A two to three-fold (depending on strain) increase in number of revertants compared to negative control, with dose-response

**Summary of individual study findings:**

Study validity: Acceptable

**Study outcome:** No strain exhibited an increased mutation rate relative to the negative control. Appropriate responses were observed with the positive controls. These data suggest DGME is not mutagenic.

**2.6.6.4.5 Study Title:** Mammalian erythrocyte micronucleus test with DGME

**Key findings:** DGME was not clastogenic under the conditions of this assay.

**Study No:** ATLS-191

**Study Type:** In vivo clastogenicity assay

**Volume # and Page #:** Mod 4, vol. 41

**Conducting Laboratory:** \_\_\_\_\_

**Date of Study Initiation:** 17-MAY-2004

**GLP Compliance:** Yes

**QA Reports Yes (X) No ( )**

**Drug, lot #, radiolabel, and % purity:** Diethylene glycol monoethyl ether (DGME), lot 021003-001. \_\_\_\_\_

**Formulation/vehicle:** DGME dissolved in water

**Methodology:**

- Strains/Species/Cell line: Mouse/ICR
- Dose Selection Criteria: Tolerability in preliminary studies
- Test Agent Stability: Chemical analyses of the test material formulations used in this study were apparently not performed
- Metabolic Activation System: NA (in vivo assay with endogenous metabolism)
- Controls:
  - Vehicle: Water
  - Negative Control: Vehicle
  - Positive Controls: Cyclophosphamide (50 mg/kg)
  - Comments: Controls were adequate
- Exposure Conditions:
  - Doses used in definitive study: 2 mL/kg of either DGME diluted 1:3 (low-dose; LD), 1:1 (mid-dose; MD), or undiluted (high-dose; HD), administered once by SC injection. Note: In a preliminary study, mortality was observed when dosages of neat DGME  $\geq$  3 mL/kg were injected.
  - Study design: 5 vehicle control, 5 positive control, 5 LD, 5 MD, and 5 HD mice per sex sacrificed 24 hours post-injection. 5 HD and 5 vehicle control mice per sex sacrificed at 48 hours post-injection. Immediately following sacrifice marrow from femurs was aspirated, centrifuged, smears produced, fixed, and stained. The slides were examined and 2000 polychromatic erythrocytes were scored for the presence of micronuclei (round, darkly staining nuclear fragments).
- Analysis:
  - Counting method: Microscope

- Genetic toxicity endpoints: Significantly increased percentage of polychromatic erythrocytes with micronuclei

**Results:**

- Study Validity: Acceptable
- Study Outcome: In main study, no unscheduled deaths occurred, although ataxia and lethargy were observed at all exposure levels, and piloerection was observed in HD animals. DGME did not increase the incidence of polychromatic erythrocytes with micronuclei. Appropriate results were obtained with the controls.

**Study Outcome:** These data suggest DGME is not clastogenic.

**2.6.6.4.6 Study Title:** Transcutol Measurement of Unscheduled DNA Synthesis in Rat Liver Using an In Vivo/In Vitro Procedure

**Key findings:** DGME was not genotoxic under the conditions of this assay.

**Study No:** TOX 96340

**Study Type:** In vivo/in vitro assay for DNA damage

**Volume # and Page #:** Mod 4, vol. 41

**Conducting Laboratory:** \_\_\_\_\_

**Date of Study Initiation:** 15-MAR-1996

**GLP Compliance:** Yes

**QA Reports** Yes (X) No ( )

**Drug, lot #, radiolabel, and % purity:** Diethylene glycol monoethyl ether (DGME), lot 9600544, —

**Formulation/vehicle:** DGME dissolved in water

**Methodology:**

- Strains/Species/Cell line: Rat/Wistar (males only)
- Dose Selection Criteria: Tolerability in preliminary studies
- Test Agent Stability: Chemical analyses of the test material formulations used in this study were apparently not performed
- Metabolic Activation System: NA (in vivo assay with endogenous metabolism)
- Controls:
  - Vehicle: Water
  - Negative Control: Vehicle
  - Positive Controls: 2-Acetamidofluorene (75 mg/kg); dimethylnitrosamine (10 mg/kg)
  - Comments: Controls were adequate
- Exposure Conditions:
  - Doses used in definitive study: 0, 800, and 2000 mg/kg as a single oral dose. Note: In a preliminary study, no mortality was observed at a "limit"

dose of 2000 mg/kg, so that was selected as the high dose for the main study.

- Study design: Groups of 5 male rats were dosed with either water (vehicle control), one of the positive control materials, or either 800 or 2000 mg/kg of DGME. Hepatocyte cultures of three animals from each group were prepared; excess animals were destroyed. Two sets of experiments were conducted. In one, the animals were sacrificed 2-4 hours post-treatment, while sacrifice occurred 12-14 hours post-treatment in the second experiment. Suspensions of isolated hepatocytes were placed in culture dishes, the cells permitted to attach to coverslips, and the cells were incubated in the presence of [<sup>3</sup>H]-thymidine. After rinsing and fixing the cells the coverslips were mounted on microscope slides and the slides dipped in photographic emulsion. The slides were stored in light proof boxes for 14 days and then developed. 100 cells per slide were examined and the numbers of nuclear and cytoplasmic grains were counted.
- Analysis:
  - Counting method: Microscope
  - Genetic toxicity endpoints: Significantly increased percentage of cells with nuclear photographic grains.

#### **Results:**

- Study Validity: Acceptable. Although toxicokinetic data were not included in the report of this study to document systemic exposure following oral dosing, a separate study documented that DGME is 79%-95% bioavailable following oral dosing of rats (study No. GAT/DIE/01001).
- Study Outcome: Under the conditions of this study, a single oral dose of DGME 2-4 or 12-14 hours prior to sacrifice did not induce hepatocellular DNA damage (cultured hepatocytes did not take up thymidine). Appropriate results were obtained with the controls.

**Study Outcome:** These data suggest DGME is not genotoxic.

### **2.6.6.5 Carcinogenicity**

**2.6.6.5.1 Study title:** 104-Week carcinogenicity study of diethylene glycol monoethyl ether and dapsone administered via oral gavage to Sprague-Dawley rats

**Key study findings:** No treatment-related effects on survival, weight gain, or histopathology were observed. No statistically significant differences in tumor incidence were observed under the conditions of this study.

Adequacy of the carcinogenicity study and appropriateness of the test model: This study was discussed by the executive carcinogenicity assessment committee on 26-APR-2005. The committee concluded that the study was minimally adequate but acceptable. The committee decided there were no clearly test-article-related neoplasms, including hemangiosarcomas and skin papillomas.

Evaluation of tumor findings: No statistically significant differences in tumor incidence were observed under the conditions of this study.

**Study no.:** 128-002

**Volume #, and page #:** Mod 4 vol 42, page 1

**Conducting laboratory and location:** \_\_\_\_\_

**Date of study initiation:** 01-FEB-2001

**GLP compliance:** Yes

**QA report:** yes ( X ) no ( )

**Drug, lot #, and % purity:** Dapsone, lot No. TN:01110D

**CAC concurrence:** Yes. Note: The protocol for the study was originally discussed by the exec-CAC on 24-OCT-2000. The sponsor contacted the division in July, 2002 (amendment 058 to IND 54,440) in regard to unexpectedly high mortality in the study, which was in week 74. Mortality was highest in the control animals. The exec-CAC recommended that the study continue, and that all members of a given gender be sacrificed when the number of surviving animals of that gender in either the control or high-dose dapsone group reaches 15, or until 104 weeks of dosing have transpired, whichever comes first. The exec-CAC requested that the sponsor be informed that the study may be deemed invalid if analysis of the final data indicated that premature mortality of control animals was abnormally high relative to historical control data. The sponsor was informed that the adequacy of the study would be determined by the exec-CAC after completion of the study and review of the final data. The exec-CAC concluded on 26-APR-2005 that the study was minimally adequate but acceptable.

**Methods**

Doses:

Group Number	Group Designation	Dosage Level (mg/kg/day)
1	Vehicle Control	0
2	DGME	540
3	Low-Dose Dapsone	1
4	Mid-Dose Dapsone	5
5	High-Dose Dapsone	15

Basis of dose selection (MTD, MFD, AUC etc.): MTD

Species/strain: Rat/Crl:CD (SD) IGS BR out-bred albino

Number/sex/group (main study): 50

Route, formulation, volume: Oral (gavage); 0.5% carboxymethylcellulose (medium viscosity) in water; 10 mL/kg

Frequency of dosing: Once daily  
 Satellite groups used for toxicokinetics or special groups: No  
 Age: Approx. 8 weeks at initiation  
 Animal housing: Individual  
 Restriction paradigm for dietary restriction studies: No  
 Drug stability/homogeneity: Acceptable  
 Dual controls employed: No  
 Interim sacrifices: No  
 Deviations from original study protocol: See note above, concerning CAC concurrence. Surviving females were sacrificed during week 92, and surviving males were sacrificed during week 100.

### Observation times

Mortality: Twice daily  
 Clinical signs: Weekly  
 Body weights: Weekly for first 13 weeks, then monthly.  
 Food consumption: Weekly for first 13 weeks, then over a one-week interval every 3 months thereafter  
 Histopathology: Yes; full range of tissues examined from groups 1, 2, and 5, all animals found dead or killed in extremis, and all gross lesions from animals in groups 3 and 4.  
 Peer review: yes ( ), no ( X )  
 Toxicokinetics: No (TK data were obtained in 90-day study)  
 Other: Complete hematology was performed at terminal sacrifice (dapsone is known to be capable of inducing hemolytic anemia).

### Results

Mortality: The survival data are presented below.

Survival Rates of Rats at Terminal Sacrifice (92 weeks for females; 100 weeks for males)

Group Number	Group Designation	Dosage Level (mg/kg/day)	Number of Males that Survived to the Terminal Sacrifice	Number of Females that Survived to the Terminal Sacrifice
1	Vehicle Control	0	15/50	13/50
2	DGME	540	24/50	27/50
3	Low-Dose Dapsone	1	17/50	21/50
4	Mid-Dose Dapsone	5	19/50	21/50
5	High-Dose Dapsone	15	15/50	22/50

Clinical signs: Clinical observations in all groups (including controls) included internal masses, skin masses, skin ulcers, reduced activity, abnormal gait, hunched posture, emaciated appearance, swollen hind limbs, unkempt appearance, and rales. These findings were considered to be unrelated to treatment, since they occurred in all groups. Transient skin discoloration (paleness) was observed in some animals, and tended to be more common with increased exposure to dapsone. The pale skin may have been secondary to dapsone-induced methemoglobinemia and/or hemolytic anemia.

Body weights: No remarkable observations. Mean body weight-gain data are summarized below:

Group Number	Group Designation	Dosage Level (mg/kg/day)	Approx. Mean BW Gain of Males, over Study Days 1-700 (grams)	Approx. Mean BW Gain of Females, over Study Days 1-645 (grams)
1	Vehicle Control	0	422	269
2	DGME	540	449	284
3	Low-Dose Dapsone	1	438	279
4	Mid-Dose Dapsone	5	439	281
5	High-Dose Dapsone	15	468	277

Food consumption: No remarkable observations.

Hematology: No remarkable observations.

Gross pathology: According to the report, the most common cause of early mortality or moribundity was pituitary adenomas. The incidence of pituitary adenomas in all animals was:

Group Number	Group Designation	Dosage Level (mg/kg/day)	Number of Males with Grossly Observed Pituitary Adenomas	Number of Females with Grossly Observed Pituitary Adenomas
1	Vehicle Control	0	9/50	26/50
2	DGME	540	5/50	13/50

3	Low-Dose Dapsone	1	11/50	24/50
4	Mid-Dose Dapsone	5	10/50	21/50
5	High-Dose Dapsone	15	10/50	16/50

Enlargement of the spleen was observed in high-dose males only.

Organ weights: In males, the mean absolute weight of the spleen of high-dose animals was significantly greater than in controls. In females, the mean weight of the thymus was significantly lower in high-dose animals, and in animals treated with DGME (groups 2 and 5). It is doubtful that these findings were toxicologically significant. No other significant differences were observed.

Histopathology:

Non-neoplastic: There were no remarkable (test article-related) histopathologic findings in any group.

Neoplastic: There were no remarkable (test article-related) histopathologic findings in any group, including no significant differences in tumor incidence.

Note: Two equivocal differences in tumor incidence were observed:

1. A trend test suggested the incidence of hemangiosarcomas in male animals increased with increasing exposure to dapsone, but a pair-wise comparison of the control and high-dose groups was negative ( $p \leq 0.0750$ ).
2. The incidence of skin papillomas in male animals appeared in a trend test to be related to exposure to dapsone, but a pair-wise comparison of the control and high-dose groups was negative ( $p \leq 0.0625$ ).

The exec-CAC decided on 26-APR-2005 that there were no clearly test-article-related neoplasms, including hemangiosarcomas and skin papillomas.

Toxicokinetics: No toxicokinetic data were obtained in this study. However, in the 90-day dose-ranging study conducted to support dosage selection for this study (study No. 128-001, reviewed above under general toxicology), the following AUC data were obtained:

Dosage Level (mg/kg/day)	AUC <sub>0-24</sub> in Males (ng·hr/mL)	AUC <sub>0-24</sub> in Females (ng·hr/mL)
3	6250	20,800
30	132,000	252,000

Extrapolating, the following approximate AUC values would be anticipated:

Dosage Level (mg/kg/day)	Estimated AUC <sub>0-24</sub> in Males (ng·hr/mL)	Estimated AUC <sub>0-24</sub> in Females (ng·hr/mL)	AUC Ratio for Males (multiple of the maximum clinical AUC value)*	AUC Ratio for Females (multiple of the maximum clinical AUC value)
1	2100	7000	5	17
5	10,400	35,000	25	84
15	66,000	126,000	159	300

\*AUC ratios are based on data from a study of acne patients that applied the drug product twice daily to 22.5% of the body surface area for 14 days, yielding an AUC<sub>0-24</sub> value of 415 ng·hr/mL.

#### 2.6.6.5.2. Study title: 26-week dermal carcinogenicity study in Tg.AC mice

**Key study findings:** Topically applied dapsone did not impact the formation of papillomas in Tg.AC mice under the conditions of this study. Topical dapsone induced a high degree of mortality. None of the mid-dose females or high-dose males or females survived to scheduled sacrifice. Left atrial thrombosis and left ventricular myocyte degeneration were observed in a dose-related manner. The thrombosis was attributed to stasis of blood within the heart due to degeneration of the myocardium. Reduced intracardiac blood flow following thrombus formation may have been responsible for the high death rate in dapsone-treated animals.

**Adequacy of the carcinogenicity study and appropriateness of the test model:** This study was discussed by the executive carcinogenicity assessment committee on 26-APR-2005. The committee concluded that this was a valid carcinogenicity assay and that no evidence of carcinogenicity had been detected.

**Evaluation of tumor findings:** No significant differences in tumor (papilloma) incidence were observed between groups.

**Study No.:** ATLS-163

**Document #, Volume #, and Page #:** Mod 4, vol. 48

**Conducting laboratory and location:** \_\_\_\_\_

**Date of study initiation:** 14-MAY-2003

**GLP compliance:** Yes

**QA report:** yes (X) no ( )

**Drug, lot #, radiolabel, and % purity:** 3% dapsone in 25% DGME gel, Lot No. 1133-114A; 5% dapsone in 25% DGME gel, Lot No. 1133-93A; 10% dapsone in 25% DGME gel, Lot No. 1133-117A; 25% DGME vehicle, Lot No. 1133-60A.

**Formulation/vehicle:** 3% dapsone in 25% DGME gel; 5% dapsone in 25% DGME gel; 10% dapsone in 25% DGME gel; negative control group received product vehicle that

contained 25% DGME; positive control group received 12-O-tetradecanoylphorbol 13-acetate (TPA) in 25% DGME gel. Additional groups received TPA in acetone, 5% dapsone in acetone, or acetone alone. DGME is diethylene glycol monoethyl ether, an excipient in the product. The experimental design is summarized below:

Group Number	Material Applied
1	25% DGME Vehicle
2	20 µg TPA in 0.1 mL 25% DGME gel
3	3% Dapsone in 25% DGME gel
4	5% Dapsone in 25% DGME gel
5	10% Dapsone in 25% DGME gel
6	Acetone
7	1.25 µg TPA in 0.1 mL acetone
8	5% Dapsone in acetone

**Methods** (unique aspects):

**Dosing:**

Species/strain: Mouse/Tg.AC (FVB/N Tac-TgN(v-Ha-ras))  
 #/sex/group or time point (main study): 25/sex/group, except 10/sex in group 7  
 Age: Approx. 7 weeks at start of dosing  
 Weight: Males: Approx. 20-30 g; Females: Approx. 17-25 g at start of dosing  
 Doses in administered units: 0% dapsone/25% DGME (vehicle); 20µg TPA in 0.1 mL 25% DGME gel; 3% dapsone/25% DGME; 5% dapsone/25% DGME; 10% dapsone/25% DGME; neat acetone; 1.25 µg TPA in 0.1 mL acetone; 5% dapsone in acetone. The positive control (TPA) groups received 0.1 mL of test material three times per week (MWF) for 26 weeks (individual animals sacrificed when 20 or more tumors were present). All other groups received a dose volume of 2 mL/kg for weeks 1-6 (groups 1 and 3-5) or weeks 1-3 (groups 6 and 8), followed by 5 mL/kg for 27 weeks. Positive control animals (groups 2 and 7) were sacrificed prior to the scheduled time if the tumor burden exceeded 20. Note: The sponsor initiated the study under a protocol that involved a dosage volume of 2 mL/kg. This was increased to 5 mL/kg, and the 27 week dosing period restarted, after the exec-CAC suggested use of a volume of 5 mL/kg.  
 Route, form, volume, and infusion rate: Topical to skin (site shaved weekly); see above for volumes used.

**Observations and times:**

Clinical signs: Animals observed twice daily for moribundity and mortality and once weekly 1-2 hours post-dosing for clinical signs of toxicity.  
 Body weights: Weekly through week 13, then every two weeks.  
 Food consumption: No

Mass palpation: Examined for skin tumors (squamous papillomas) weekly, and the numbers of tumors within the application site and outside the application area were recorded.

Clinical pathology: Full hematology on 5 animals per group at termination.

Toxicokinetics: None

Gross pathology: All animals.

Organ weights: Brain, heart, liver, kidneys, lungs, thymus, spleen, testes/ovaries.

Histopathology: For all animals in groups 1, 3, 4, 5, 6, 8 (excluding positive controls): non-lesioned skin from treatment site, skin sample from site other than treatment site, non-skin-tumor gross lesions, and the heart, spleen, pituitary, thyroid, liver, and ovaries. For all animals in all groups, all the skin tumors that were identified during the in-life phase of the study, that were located within the area of application, and that could be located during necropsy, were histologically examined.

### Results:

Mortality: Unscheduled deaths (either found dead or sacrificed in extremis) are summarized below:

Group Number	Material Applied	No. of Unscheduled Male Deaths	No. of Unscheduled Female Deaths
1	25% DGME Vehicle	1/25	4/25
2	20 µg TPA in 0.1 mL 25% DGME gel	NA*	NA*
3	3% Dapsone in 25% DGME gel	3/25	6/25
4	5% Dapsone in 25% DGME gel	7/25	25/25
5	10% Dapsone in 25% DGME gel	25/25	25/25
6	Acetone	1/25	3/25
7	1.25 µg TPA in 0.1 mL acetone	NA*	NA*
8	5% Dapsone in acetone	10/25	22/25

\*Per protocol, all positive control animals sacrificed when tumor burden reached 20.

Clinical signs: Observations of rapid and shallow respiration, ruffled fur, stained coats, and thin appearance tended to increase with increased exposure to dapsone. Pallor, lethargy, prostration, and dyspnea were observed in some animals in the high-dose group (group 5) only.

Dermal irritation: Observed at application site of two males each from groups 2, 4, and 5, and in four group 5 females.

Body weight gains: Mean body weight gain was significantly reduced over certain intervals in group 4 and 5 (mid and high-dose) animals of both genders.

Hematology: Erythrocytic parameters, including RBC count and HCT, tended to be reduced (in some cases significantly) in all groups that received dapsone.

Clinical chemistry: NA

Urinalysis: NA

Organ Weights: Mean absolute spleen and heart weights tended to be slightly increased in animals treated with dapsone.

Gross pathology: Abnormal gross observations in animals treated with dapsone included enlarged spleens, atria, and pituitaries, thin carcasses, and skin lesions (tumors).

Histopathology: Treatment-related findings included:

Heart: Left atrial thrombosis and left ventricular myocyte degeneration were observed in a dose-related manner. The thrombosis was attributed to stasis of blood within the heart due to degeneration of the myocardium. The pathology report suggested that reduced intracardiac blood flow following thrombus formation was responsible for the high death rate in dapsone-treated animals.

Thyroid: Follicular cell hyperplasia was observed in the thyroid glands of 1/25 group 3 (low-dose) males, 19/24 group 4 (mid-dose) males, 25/25 group 5 (high-dose) males, and 22/25 group 8 (dapsone in acetone) males, and, in females, in 19/24 in group 3, 23/23 group in group 4, 21/22 in group 5, and 25/25 in group 8. No follicular hyperplasia was observed in control animals. Sulfonamides, such as dapsone, are known to be goitrogenic by interfering with synthesis of thyroid hormone. The incidence of follicular cell adenomas tended to be above the level in control groups in groups of both genders that were exposed to dapsone:

Group Number	Material Applied	No. of Males with Follicular Cell Adenomas	No. of Females with Follicular Cell Adenomas
1	25% DGME Vehicle	0/24	0/24
2	20 µg TPA in 0.1 mL 25% DGME gel	NA	NA
3	3% Dapsone in 25% DGME gel	0/25	5/24
4	5% Dapsone in 25% DGME gel	1/24	2/23
5	10% Dapsone in 25% DGME gel	4/25	2/22
6	Acetone	0/25	0/24
7	1.25 µg TPA in 0.1 mL acetone	NA	NA
8	5% Dapsone in acetone	5/25	5/25

Pituitary: The incidence of hypertrophy of the pars distalis was observed in proportion to exposure to dapsons, presumably as a result of the goitrogenic effect of sulfonamides noted above.

Skin: No significant differences in papilloma incidence (numbers of animals bearing at least one papilloma) were apparent between groups treated with dapsons gel (groups 3, 4, and 5) and the control group (group 1). Positive control groups (groups 2 and 7) did exhibit a significantly increased incidence of animals with at least one papilloma relative to the respective negative control groups (groups 1 and 6). Papilloma incidences are summarized in the tables below:

**TABLE 11 - SUMMARY OF INCIDENCE OF ANIMALS WITH PAPILOMAS**

Arix 26-WEEK STUDY AA52LE.7D92.BTL -- PAPILOMA DATA

L=Latent papilloma not yet observed for 3 weekly observations

A=Maximum Actual papilloma observed for 3 weekly observations

AD=Maximum Actual papilloma observed for 3 weekly observations but subsequently disappeared

SOA= Site of Application

NSOA=Non-SOA

(Groups 1-5)	DGME PORTION STUDY WEEK 33
(Groups 6-8)	ACETONE PORTION STUDY WEEK 31

Group No.	Sex (M or F)	Incidence (Latent)			Incidence (Actual)		
		Animals bearing at least one latent papilloma			Animals bearing at least one actual papilloma		
		per effective number of Animals (% incidence)			per effective number of Animals (% incidence)		
		SOA	NSOA	Anywhere	SOA	NSOA	Anywhere
1	M	1/24 (4)	1/24 (4)	2/24 (8)	0/24 (0)	1/24 (4)	1/24 (4)
1	F	0/21 (0)	1/21 (5)	1/21 (5)	1/21 (5)	5/21 (24)	6/21 (29)
2	M	0/25 (0)	0/25 (0)	0/25 (0)	24/25 (96)+	13/25 (52)	24/25 (96)
2	F	2/25 (8)	0/25 (0)	2/25 (8)	24/25 (96)+	10/25 (40)	24/25 (96)
3	M	0/25 (0)	0/25 (0)	0/25 (0)	0/25 (0)	0/25 (0)	0/25 (0)
3	F	2/23 (9)	0/23 (0)	2/23 (9)	0/23 (0)	8/23 (35)	8/23 (35)
4	M	0/21 (0)	0/21 (0)	0/21 (0)	1/21 (5)	0/21 (0)	1/21 (5)
4	F	0/24 (0)	0/24 (0)	0/24 (0)	1/24 (4)	1/24 (4)	2/24 (8)

Effective number of animals = number of animals surviving per sex per group at the time of appearance of the first SOA tumor in that group.  
 Statistical Analysis (Fisher's Exact test); + = p < 0.05 (Statistical analysis performed for SOA incidences only)

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TABLE 11 - SUMMARY OF INCIDENCE OF ANIMALS WITH PAPILLOMAS - CONTINUED

Atix 26-WEEK STUDY AA52LE.7D82.BTL - PAPILOMA DATA

L=Latent papilloma not yet observed for 3 weekly observations  
 A=Maximum Actual papilloma observed for 3 weekly observations  
 AD=Maximum Actual papilloma observed for 3 weekly observations but subsequently disappeared  
 SOA= Site of Application  
 NSOA=Non-SOA

(Groups 1-5)	DGME PORTION STUDY WEEK 33
(Groups 6-8)	ACETONE PORTION STUDY WEEK 31

Group No.	Sex (M or F)	Incidence (Latent)			Incidence (Actual)		
		Animals bearing at least one latent papilloma per effective number of Animals (% incidence)			Animals bearing at least one actual papilloma per effective number of Animals (% incidence)		
		SOA	NSOA	Anywhere	SOA	NSOA	Anywhere
5	M	0/24 (0)	0/24 (0)	0/24 (0)	0/24 (0)	0/24 (0)	0/24 (0)
5	F	0/0* (**)	0/0* (**)	0/0* (**)	0/0* (**)	3/6* (**)	3/0* (**)
6	M	0/25 (0)	2/25 (8)	2/25 (8)	1/25 (4)	2/25 (8)	3/25 (12)
6	F	0/22 (0)	1/22 (5)	1/22 (5)	0/22 (0)	1/22 (5)	1/22 (5)
7	M	3/10 (30)*	1/10 (10)	3/10 (30)	10/10 (100)*	3/10 (30)	10/10 (100)
7	F	1/10 (10)	0/10 (0)	1/10 (10)	9/10 (90)*	2/10 (20)	9/10 (90)
8	M	0/15 (0)	1/15 (7)	1/15 (7)	0/15 (0)	3/15 (20)	3/15 (20)
8	F	0/25 (0)	0/25 (0)	0/25 (0)	0/25 (0)	0/25 (0)	0/25 (0)

Effective number of animals = number of animals surviving per sex per group at the time of appearance of the first SOA tumor in that group.

\* All animals died prior to terminal sacrifice with no SOA tumors  
 \*\* Can not be calculated

Statistical Analysis (Fisher's Exact test): + = p < 0.05 (Statistical analysis performed for SOA incidences only)

TABLE 11 - SUMMARY OF INCIDENCE OF ANIMALS WITH PAPILLOMAS - CONTINUED

Atix 26-WEEK STUDY AA52LE.7D82.BTL - PAPILOMA DATA

L=Latent papilloma not yet observed for 3 weekly observations  
 A=Maximum Actual papilloma observed for 3 weekly observations  
 AD=Maximum Actual papilloma observed for 3 weekly observations but subsequently disappeared  
 SOA= Site of Application  
 NSOA=Non-SOA

(Groups 1-5)	DGME PORTION STUDY WEEK 33
(Groups 6-8)	ACETONE PORTION STUDY WEEK 31

Group No.	Sex (M or F)	Incidence (All)		
		Animals bearing at least one actual or latent papilloma per effective number of Animals (% incidence)		
		SOA	NSOA	Anywhere
1	M	1/24 (4)	2/24 (8)	3/24 (13)
1	F	1/21 (5)	8/21 (29)	7/21 (33)
2	M	24/25 (96)*	13/25 (52)	24/25 (96)
2	F	24/25 (96)*	10/25 (40)	24/25 (96)
3	M	0/25 (0)	0/25 (0)	0/25 (0)
3	F	2/23 (9)	8/23 (35)	9/23 (39)
4	M	1/21 (5)	0/21 (0)	1/21 (5)
4	F	1/24 (4)	1/24 (4)	2/24 (8)

Effective number of animals = number of animals surviving per sex per group at the time of appearance of the first SOA tumor in that group.

Statistical Analysis (Fisher's Exact test): + = p < 0.05 (Statistical analysis performed for SOA incidences only)

TABLE 11 - SUMMARY OF INCIDENCE OF ANIMALS WITH PAPILOMAS - CONTINUED

Airix 26-WEEK STUDY AA52LE.7D82.BTL -- PAPILOMA DATA

L=Latent papilloma not yet observed for 3 weekly observations  
 A=Maximum Actual papilloma observed for 3 weekly observations  
 AD=Maximum Actual papilloma observed for 3 weekly observations but subsequently disappeared  
 SOA= Site of Application  
 NSOA=Non-SOA

		Incidence (All)		
		Animals bearing at least one actual or latent papilloma per effective number of Animals (% Incidence)		
Group No.	Sex (M or F)	SOA	NSOA	Anywhere
		DGME PORTION STUDY WEEK 33		
		ACETONE PORTION STUDY WEEK 31		
5	M	0/24 (0)	0/24 (0)	0/24 (0)
5	F	0/0* (**)	3/0* (**)	3/0* (**)
6	M	1/25 (4)	3/25 (12)	4/25 (16)
6	F	0/22 (0)	2/22 (9)	2/22 (9)
7	M	10/10 (100)+	3/10 (30)	10/10 (100)
7	F	9/10 (90)+	2/10 (20)	9/10 (90)
8	M	0/15 (0)	3/15 (20)	3/15 (20)
8	F	0/25 (0)	0/25 (0)	0/25 (0)

Effective number of animals = number of animals surviving per sex per group at the time of appearance of the first SOA tumor in that group.

\* All animals died prior to terminal sacrifice with no SOA tumors

\*\* Can not be calculated

Statistical Analysis (Fisher's Exact test): + = p < 0.05 (Statistical analysis performed for SOA incidences only)

- Toxicokinetics: Although toxicokinetic data were not obtained in the pivotal Tg.AC assay, the following data were obtained in the 30-day study conducted to support dosage selection for the pivotal study (the 30-day study was No. ATLS-171, conducted in FVB/N mice):

ATLS-171 Toxicokinetic Report

Table 1. Mean Plasma Concentrations of Dapsone in Mice Receiving Dapsone in Acetone or 25% DGME

Time (hr)	Mean Plasma Dapsone Concentration (µg/mL)					
	3% Dapsone in Acetone		5% Dapsone in Acetone		10% Dapsone in Acetone	
	Males Mean ± SD	Females Mean ± SD	Males Mean ± SD	Females Mean ± SD	Males Mean ± SD	Females Mean ± SD
0.5	7.15 ± 2.26	10.67 ± 1.86	17.80 ± 4.45	35.33 ± 19.45	23.30 ± 2.65	25.03 ± 0.38
1	14.07 ± 2.66	19.77 ± 4.21	16.53 ± 7.51	26.30 ± 4.68	22.60 ± 11.10	34.63 ± 2.60
2	18.43 ± 5.01	17.51 ± 6.80	29.75 ± 9.25	32.40 ± 3.38	31.87 ± 7.40	34.55 ± 3.15
4	22.57 ± 6.18	25.70 ± 0.36	29.07 ± 8.14	33.80 ± 3.60	28.57 ± 5.95	35.23 ± 4.39
8	10.03 ± 6.42	25.60 ± 4.12	28.70 ± 6.03	23.90 ± 11.35	43.85 *	29.05 ± 6.87
24	3.84 ± 1.88	5.45 ± 0.58	7.88 ± 3.49	17.37 ± 2.66	13.00 *	23.07 ± 3.95
Time (hr)	3% Dapsone in 25% DGME				10% Dapsone in 25% DGME	
	Males Mean ± SD	Females Mean ± SD			Males Mean ± SD	Females Mean ± SD
0.5	3.95 ± 1.94	13.13 ± 2.87			10.10 ± 5.75	15.33 ± 1.04
1	8.98 ± 2.58	14.47 ± 1.52			15.37 ± 3.32	35.60 ± 7.66
2	9.62 ± 2.37	21.23 ± 4.51			18.67 ± 5.95	30.70 ± 10.91
4	12.06 ± 2.06	26.97 ± 12.73			20.10 ± 4.62	41.17 ± 4.35
8	14.38 ± 4.62	29.00 ± 3.16			33.97 ± 6.65	36.57 ± 12.24
24	1.64 ± 0.62	4.03 ± 0.63			6.51 ± 1.17	16.43 ± 4.48

n = 3, except as noted. SD not calculated for n < 3.

\* n = 2.

Table 2. Pharmacokinetic Parameters for Mice Receiving Dapsone in Acetone or 25% DGME

Parameter	3% Dapsone in Acetone		5% Dapsone in Acetone		10% Dapsone in Acetone	
	Males	Females	Males	Females	Males	Females
C <sub>max</sub> (µg/mL)	22.6	25.7	29.7	35.3	43.9	35.2
T <sub>max</sub> (hr)	4	4	2	0.5	8	4
AUC <sub>0-24</sub> (µg•hr/mL)	241	425	505	570	708	677
Parameter	3% Dapsone in 25% DGME				10% Dapsone in 25% DGME	
	Males	Females			Males	Females
C <sub>max</sub> (µg/mL)	14.4	29.0			34.0	41.2
T <sub>max</sub> (hr)	8	8			8	4
AUC <sub>0-24</sub> (µg•hr/mL)	217	453			498	705

Based upon those data the following AUC ratios were calculated:

Concentration of Dapsone Applied (% w/w)	AUC Ratio for Males (multiple of the maximum clinical AUC value)*	AUC Ratio for Females (multiple of the maximum clinical AUC value)
3	520	1090
5	870**	1820
10	1200	1700

\*AUC ratios are based on data from a study of acne patients that applied the drug product twice daily to 22.5% of the body surface area for 14 days, yielding an AUC<sub>0-24</sub> value of 415 ng•hr/mL.

\*\*Based upon AUC estimate extrapolated from 3% dapsone group data.

**2.6.6.5.3. Study title:** 12-Month topical study to determine the influence of dapsone in a diethylene glycol monoethyl ether-based formulation on photocarcinogenesis in hairless mice

**Key study findings:** The product vehicle did not impact UV-induced tumor formation. The median time to first tumor greater than or equal to 1 mm for groups treated with dapsone was significantly greater than in the vehicle treated control group, suggesting that the dapsone-containing formulations inhibited UVR-induced tumor formation.

**Study No.:** ATLS-122

**Document #, Volume #, and Page #:** Mod 4, vol 52

**Conducting laboratory and location:** \_\_\_\_\_

Best Available Copy

**Date of study initiation:** 22-AUG-2000

**GLP compliance:** Yes

**QA report:** yes ( X ) no ( )

**Drug, lot #, radiolabel, and % purity:** 1% dapsone in 10% DGME gel, Lot No. SL753-42; 3% dapsone in 17.5% DGME gel, Lot No. SL753-103, 5% dapsone in 25% DGME gel, Lot No. SL753-40, 25% DGME vehicle, Lot No. SL753-104.

**Formulation/vehicle:** 1% dapsone in 10% DGME gel, 3% dapsone in 17.5% DGME gel, 5% dapsone in 25% DGME gel, and 25% DGME vehicle gel were tested. Each of the materials contained / % (w/w) methylparaben, / % carbomer 980, / % NaOH, and enough water to bring the formulation to / %.

**Methods (unique aspects):**

**Dosing:**

Species/strain: Mouse/Crl:SKH1-hrBR (albino hairless)

#/sex/group or time point (main study): 36/sex/group; housed 1 per cage

Satellite groups used for toxicokinetics or recovery: No

Age: Approximately 60 days at initiation

Weight: At study assignment: males, 26-34 g, females, 21-27 g

Doses in administered units:

Group	Dapsone Exposure (mg/kg/day)*	Dapsone Concentration in Gel (% w/w)	Volume of Test Material Applied Per Day (µL/mouse)
1	0	0	50
2	20	1	50
3	60	3	50
4	100	5	50
5	0	NA**	0
6	0	NA**	0

\*Approximate, based upon assumed BW of 25 g.

\*\*No test material applied to these animals.

The test materials were applied, and the mice irradiated, five days per week (M-F) for 40 weeks. The test materials were applied approximately 70 minutes prior to UVR exposure on Mondays, Wednesdays, and Fridays, and approximately 70 minutes following UVR exposure on Tuesdays and Thursdays. The UVR exposure was 120 RBU per day (600 RBU per week) for all groups except group 6, which received 240 RBU per day (1200 RBU per week). UV light was generated by a 6.5 kW long arc lamp with a 1 mm filter and with definitive output in both the UVA (320 nm to 400 nm) and UVB (280 nm to 320 nm) ranges.

All surviving animals were maintained for 12 weeks without treatment following 40 weeks of treatment, with sacrifice during week 52. Mice were sacrificed prematurely if a skin tumor  $\geq 10$  mm diameter was present. All mice in a given

dosage/gender group were killed: a) when survival in that group reached 50%; and b) if more than 50% of the surviving mice had tumors  $\geq 4$  mm diameter.

Route, form, volume, and infusion rate: Topical, 50  $\mu$ L/day (see above), once per day M-F for 40 consecutive weeks. The assigned material was applied to the back and sides (approximately 25 cm<sup>2</sup>) of the mice.

**Observations and times:**

Clinical signs: Animals observed twice daily for viability and weekly for general appearance. Clinical signs and local skin reactions (including skin tumors) weekly.  
Body weights: Weekly for first 13 weeks, then every four weeks

Food consumption: No

Hematology: No

Clinical chemistry: No

Urinalysis: No

Gross pathology: All animals

Organs weighed: None

Histopathology: No

Toxicokinetics: No

**Results:**

Survival: No drug-related effects on survival were observed. Increased exposure to UVR resulted in an increased rate of mortality (due to increased tumor burden).

Numbers of Animals Surviving to Scheduled Sacrifice

Group	Dapsone Exposure (mg/kg/day)*	UVR Exposure (RBU/Week)	Number of Males Killed at Scheduled Sacrifice, Week 53	Number of Females Killed at Scheduled Sacrifice, Week 53
1	0 (Vehicle)	600	28/36	29/36
2	20	600	28/36	29/36
3	60	600	31/36	30/36
4	100	600	35/36	27/36
5	0 (No treatment)	600	24/36	23/36
6	0 (No treatment)	1200	0/36**	0/36**

\*Approximate, based upon assumed BW of 25 g.

\*\*All group 6 animals died or were sacrificed due to excessive tumor burden between approximately weeks 30 and 45.

Clinical signs: All test materials were well tolerated, although some edema and flaking were observed. The incidence of edema and flaking were significantly reduced in groups 2-4 compared to group 1.

Body weights: Treatment had no remarkable effects on mean body weights or weight gains (female mice in groups 3 and 4 appeared to gain slightly more weight than did group 1 animals, but this was probably not toxicologically relevant):

Body weight gains (mean±SD):

Group	Dapsone Exposure (mg/kg/day)*	UVR Exposure (RBU/Week)	Mean BW Change for Males, Weeks 1-53	Mean BW Change for Females, Weeks 1-53
1	0 (Vehicle)	600	10.4±3.4	11.2±3.4
2	20	600	9.8±3.8	10.8±2.6
3	60	600	10.5±2.5	12.4±3.2
4	100	600	10.6±2.4	13.1±2.6**
5	0 (No treatment)	600	10.6±1.7	11.9±2.8
6	0 (No treatment)	1200	NA	NA

\*Approximate, based upon assumed BW of 25 g.

\*\*p<0.05

Gross pathology: No remarkable observations, with exception of skin tumors (see below).

Tumor data analysis: Data concerning the median number of weeks on study at which the first tumor (for a given animal) greater than or equal to 1.0 mm in diameter was observed are summarized below:

Median Number of Weeks on Study at Which First Tumor  $\geq$  1 mm Diameter was Observed:

Group	Dapsone Exposure (mg/kg/day)*	UVR Exposure (RBU/Week)	Median Week to Tumor $\geq$ 1 mm, Males	Median Week to Tumor $\geq$ 1 mm, Females
1	0 (Vehicle)	600	45.3	39.5
2	20	600	50.0**	45.0**
3	60	600	53.0***	45.5**
4	100	600	53.0***	49.0***
5	0 (No treatment)	600	44.0	39.5
6	0 (No treatment)	1200	25.0****	24.0****

\*Approximate, based upon assumed BW of 25 g.

\*\*p<0.01 compared to group 1.

\*\*\*p<0.001 compared to group 1.

\*\*\*\*p<0.001 compared to group 5.

The median time to first tumor greater than or equal to 1 mm for groups 1 and 5 did not differ statistically. The only difference between groups 1 and 5 was that group 1 animals received vehicle plus 600 RBU per week, while group 5 animals

received only 600 RBU per week. These data suggest that the product vehicle did not impact UV-induced tumor formation.

The median time to first tumor greater than or equal to 1 mm for groups 2, 3, and 4 was significantly greater than in group 1 (vehicle treated control), suggesting that the dapsone-containing formulations inhibited UVR-induced tumor formation.

The median time to first tumor greater than or equal to 1 mm tended to be longer in males than in females, achieving statistical significance for comparisons between the genders for groups 1, 2, 3, and 5.

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#### 2.6.6.6 Reproductive and developmental toxicology

##### Fertility and early embryonic development

**2.6.6.6.1 Study title:** Dapsone and diethylene glycol monoethyl ether: Oral (gavage) fertility and general reproduction toxicity study in male rats

**Key study findings:** Dapsone impaired fertility of male rats. This was evident from reduction in the fertility index (number of rats pregnant/number of rats mated), reduced sperm motility (percentage of observed sperm that were motile), and reduced numbers of implantations and viable embryos in the females that did become pregnant. Statistically significant reductions in percentage of motile sperm were observed at exposures of 3 mg/kg/day and above. 0.5 mg/kg/day was an apparent NOAEL in this study, although a nonsignificant trend toward a reduction in the percentage of motile sperm may have been apparent. DGME had no effects in this study.

**Study no.:** ATLS-119

**Volume #, and page #:** Mod 4, vol 56

**Conducting laboratory and location:** \_\_\_\_\_

**Date of study initiation:** 11-APR-2000

**GLP compliance:** Yes

**QA reports:** yes ( X ) no ( )

**Drug, lot #, and % purity:** Dapsone, USP (powder), lot No. 01110C, \_\_\_\_\_% pure; DGME, lot No. 11189A, \_\_\_\_\_% pure; carboxymethylcellulose, lot Nos. 69H0028 and 20K0237 (used as vehicle for dapsone and DGME as a 0.5% aqueous solution)

##### Methods

Doses: 0, 0.5, 3, 12, 30, and 75 mg/kg/day of dapsone, and 180 mg/kg/day of DGME. Note: The study was conducted in two stages, with an initial study that

involved dosages of 0, 12, 30, and 75 mg/kg/day, and a second study that involved exposures of 0, 0.5, 3, and 12 mg/kg/day. Therefore, there were two groups of animals that received 0 mg/kg/day (vehicle control groups) and two groups that received 12 mg/kg/day.

Species/strain: Rat/Crl:CD(SD)IGS BR VAF/Plus

Number/sex/group: 25 (only males were treated; untreated females were used to confirm male reproductive potential)

Route, formulation, volume, and infusion rate: Oral (gavage), dapsone or DGME dissolved/suspended in 0.5% CMC solution.

Satellite groups used for toxicokinetics: No

Study design: F0 male rats were dosed with test materials beginning 63 days prior to cohabitation with untreated females and continuing through the day prior to sacrifice.

Parameters and endpoints evaluated: F0 males were observed for viability and clinical signs twice daily. Body weight was recorded daily. F0 females were monitored for body weight and clinical signs on gestation days 0, 7, 10, and 13. All F0 females were sacrificed on gestation day 13, cesarean-sectioned, gross necropsied, and the numbers of corpora lutea, implantation sites, and viable and non-viable embryos was recorded. Following completion of the cohabitation period, F1 males were subjected to gross necropsy, the reproductive organs (each testis, each epididymis, seminal vesicles, and prostate) weighed, and sperm concentration and motility were evaluated.

## Results

Mortality: No remarkable unscheduled deaths.

Clinical signs: Treatment-related observations included pale or blue extremities, salivation, and poor grooming; the incidence of these findings was proportional to exposure. No effect in DGME group.

Body weight: Body weight gain was significantly reduced at 75 mg/kg/day (high-dose group); the terminal body weight of males treated at 75 mg/kg/day was significantly lower than that of controls (530.4±39.5 g compared to 574.4±59.6 g, p<0.01). No other remarkable effects. No effect in DGME group.

Food consumption: No remarkable observations.

Toxicokinetics: NA

Necropsy: Enlarged spleen was observed in F0 males at 12 mg/kg/day and above. The mean absolute weights of the right and left epididymis, and of the left cauda epididymis were significantly reduced in F0 males at 12 mg/kg/day or greater. No effects in DGME group.

Fertility parameters (mating/fertility index, corpora lutea, preimplantation loss, etc.):

F0 males: The fertility index (number of rats pregnant/number of rats mated) was significantly reduced ( $p < 0.01$ ) at 75 mg/kg/day (19/25 compared to 23/25 in the control group, or 76% vs. 92%, in the high-dose and control groups, respectively). No other significant differences or trends were observed, including no effects on average time for mating to occur or on the number of rats that mated (produced a semen plug) within a group.

The percentage of sperm that exhibited motility was significantly reduced in proportion to exposure to dapsone, as summarized below:

Main study

Group	Total Sperm Count <sup>1</sup>	% Motile
1 (vehicle)	488.5±223.0	77.1±22.1
2 (DGME)	387.8±157.8	79.0±18.8
3 (12 mg/kg/day)	389.8±139.4	53.7±21.6**
4 (30 mg/kg/day)	409.9±160.4	34.1±17.2**
5 (75 mg/kg/day)	400.8±173.4	31.6±19.5**

<sup>1</sup>Number of sperm observed when groups of 5 fields were examined until either 200 sperm were counted or 20 fields had been observed.

\*\* $p < 0.01$

Follow-up study

Group	Total Sperm Count <sup>1</sup>	% Motile
1 (vehicle)	366.4±159.5	88.7±4.6
2 (0.5 mg/kg/day)	368.2±119.5	78.0±21.9
3 (3 mg/kg/day)	378.9±118.2	63.1±21.7**
4 (12 mg/kg/day)	384.8±106.9	46.6±19.5**

<sup>1</sup>Number of sperm observed when groups of 5 fields were examined until either 200 sperm were counted or 20 fields had been observed.

\*\* $p < 0.01$

The report stated that the "sperm density" (the number of sperm counted in 10 fields, divided by the volume in the image area, and correcting for dilution of the sample) was significantly lower in males treated with 3 and 12 mg/kg/day in the follow-up study. However, there was no clear effect on this parameter in the main study, even at exposures as high as 75 mg/kg/day, so I discounted this finding.

No effects in DGME group.

F0 females: The mean numbers of implantations and viable embryos were significantly reduced in females mated with males that had been dosed at 12 mg/kg/day or greater, presumably due to reduced numbers or effectiveness of sperm.

**2.6.6.6.2 Study title:** Oral (gavage) fertility and general reproduction toxicity study and recovery study of dapsone in male rats, study No. ATLS-183.

**Key study findings:** Dapsone did not impair fertility of male rats at the exposure levels examined in this study (0.25, 0.5, 1, and 2 mg/kg/day).

**Study no.:** ATLS-183

**Volume #, and page #:** Mod 4, vol 57

**Conducting laboratory and location:** \_\_\_\_\_

**Date of study initiation:** 11-NOV-2003

**GLP compliance:** Yes

**QA reports:** yes ( X ) no ( )

**Drug, lot #, and % purity:** Dapsone, USP (powder), lot No. 101001-000, \_\_\_\_\_% pure; carboxymethylcellulose, lot Nos. 120K0252 and 61K0014 (used as vehicle for dapsone and DGME as a 0.5% aqueous solution)

#### Methods

Doses: 0, 0.25, 0.5, 1, and 2 mg/kg/day of dapsone.

Species/strain: Rat/Crl:CD(SD)IGS BR VAF/Plus

Number/sex/group: 40 (only males were treated; untreated females were used to confirm male reproductive potential). Note: 20 males per group were sacrificed following the last dose, 10 males per group were sacrificed following a four week recovery period, and 10 males per group were sacrificed following a ten week recovery period.

Route, formulation, volume, and infusion rate: Oral (gavage), dapsone dissolved/suspended in 0.5% CMC solution.

Satellite groups used for toxicokinetics: No, but two recovery groups (see above).

Study design: F0 male rats were dosed with test materials beginning 63 days prior to cohabitation with untreated females and continuing through the day prior to sacrifice (doses administered on approximately 100 consecutive days).

Parameters and endpoints evaluated: F0 males were observed for viability and clinical signs twice daily. Body weight was recorded daily during the dosing period, then weekly for recovery animals. F0 females were monitored for body weight and clinical signs on gestation days 0, 7, 10, and 13. All F0 females were sacrificed on gestation day 13, cesarean-sectioned, gross necropsied, and the numbers of corpora lutea, implantation sites, and viable and non-viable embryos was recorded. Following completion of the cohabitation period, F1 males were subjected to gross necropsy, the reproductive organs (each testis, each epididymis, seminal vesicles, and prostate) weighed, and sperm concentration and motility were evaluated. Blood samples were obtained from 3 males per treatment group on days 1, 28, and the last day of dosing immediately prior to dosing and at 0.5, 1, 2, 4, 8, and 24 hours post-treatment. These samples were analyzed for dapsone concentration.

**Results**

Mortality: No remarkable unscheduled deaths.

Clinical signs: No remarkable observations.

Body weight: No remarkable observations.

Food consumption: No remarkable observations.

Toxicokinetics:

Time Point	Dose (mg/kg)	C <sub>max</sub> (ng/mL)	T <sub>max</sub> (hr)	AUC <sub>0-24</sub> (ng·hr/mL)
Day 1	0.25	79.8	1	492
	0.5	127.5	1	880
	1	299.0	0.5	1820
	2	524.7	1	3749
Day 28	0.25	87.0	0.5	452
	0.5	160.7	1	960
	1	358.3	0.5	1846
	2	562.3	0.5	3422
Last Dose	0.25	99.2	0.5	551
	0.5	221.3	0.5	1061
	1	443.3	0.5	2303
	2	673.0	0.5	4469

Necropsy: No remarkable observations.

Fertility parameters (mating/fertility index, corpora lutea, preimplantation loss, etc.):

F0 males: No significant differences or trends were observed, including no effects on average time for mating to occur, the number of rats that mated (produced a semen plug) within a group, or the percentage of males that successfully impregnated a female (fertility index).

The percentage of sperm that exhibited motility was statistically significantly reduced in males that received 2 mg/kg/day, although examination of the values below suggests this was not a genuine, treatment-related, effect:

Group	Total Sperm Count <sup>1</sup>	% Motile
1 (vehicle)	387.7±137.9	93.7±2.9
2 (0.25 mg/kg/day)	361.4±103.0	94.0±3.2
3 (0.5 mg/kg/day)	331.6±112.8	85.9±14.3

4 (1 mg/kg/day)	344.5±92.2	91.6±5.7
5 (2 mg/kg/day)	319.4±93.6	85.8±13.3*

<sup>1</sup>Number of sperm observed when groups of 5 fields were examined until either 200 sperm were counted or 20 fields had been observed.

\*p<0.05

No significant differences were observed in sperm parameters in either recovery group.

F0 females: No remarkable observations, including no significant differences in numbers of implantations or numbers of viable and nonviable embryos.

### Embryofetal development

Note: The following study served as both a rodent teratology study and a female fertility study.

**2.6.6.6.3 Study title:** Dapsone and diethylene glycol monoethyl ether: combined oral (gavage) fertility and developmental toxicity study in female rats

**Key study findings:** When administered to female rats at a dosage of 75mg/kg/day for 15 days prior to mating and for 17 days thereafter, dapsone reduced the mean number of implantations, increased the mean early resorption rate, and reduced the mean litter size. These effects were probably secondary to maternal toxicity. No effects on the incidence of external, visceral or skeletal malformations or variations were observed. Under the conditions of this study, the NOAELs for dapsone and DGME were 12mg/kg/day and 180mg/kg/day, respectively. DGME did not induce toxicity under the conditions of this study.

**Study no.:** ATLS-120

**Volume #, and page #:** Mod 4, vol 55

**Conducting laboratory and location:** \_\_\_\_\_

**Date of study initiation:** 7-APR-2000

**GLP compliance:** Yes

**QA reports:** yes (X) no ( )

**Drug, lot #, radiolabel, and % purity:** Dapsone, USP, Lot #01110C; Diethylene glycol monoethyl ether (DGME), Lot#11189A

**Formulation/vehicle:** The test materials were prepared as suspensions of dapsone or solutions of DGME. 0.5% carboxymethylcellulose in purified deionized water was used as the vehicle.

#### Methods:

Species/strain: Rat/Crl:CD(SD)IGS BR VAF/Plus

Doses employed: 0 (control), 12, 30, and 75mg/kg/day dapsone; a satellite group received 180mg/kg/day DGME (an excipient in the product)

Route of administration: Oral (gavage) once daily

Study design: Virgin females were paired with males; day on which mating was confirmed (plug or sperm in vagina) was designated day 0. Females only were dosed, beginning 15 days prior to pairing and continuing through day 17 of gestation. Dams were killed on day 21 and C-sectioned.

Number/group: 25

Parameters and endpoints evaluated: Maternal survival and body weight.

Numbers of live, dead, and resorbed fetuses were determined. Live fetuses were weighed and examined for external, visceral, and skeletal anomalies.

### Results:

In-life (maternal) observations:

Maternal Mortality: None

Clinical signs: Increased licking, sniffing, chewing, and salivation at 75mg/kg/day

Maternal body weight: Significantly reduced body weight gain throughout period of dosing at 30mg/kg/day and above.

Food consumption: Slightly reduced at 30mg/kg/day and above during treatment period; increased at 30mg/kg/day and above days 18-21 (after cessation of treatment).

Toxicokinetics: NA

Note: No effects on latency to mate or fertility index

Terminal and necroscopic evaluations (offspring):

Mean implantations: Reduced at 75mg/kg/day; note that this is presumably related to treatment. Unlike most teratology studies, F0 females were dosed for 15 days prior to pairing.

Body weight of live fetuses (male/female combined; grams, mean±SD): No remarkable observations

No. of Live fetuses at C-section (expressed as percentage of implantations): Slightly reduced at 30mg/kg/day; significantly reduced at 75mg/kg/day.

No. of early resorptions: Significantly increased at 75mg/kg/day only.

No. of late resorptions: No remarkable observations

Gross non-skeletal anomalies: No remarkable observations

Skeletal anomalies: No remarkable observations

**2.6.6.6.4 Study title:** Dapsone and diethylene glycol monoethyl ether: oral (stomach tube) developmental toxicity study in rabbits

**Key study findings:** When administered at a dosage of 150 mg/kg/day to rabbits on days 6-18 of gestation, dapsone significantly increased the incidence of early resorptions. Two does at this dosage delivered prematurely and seven does resorbed all fetuses. These effects were probably secondary to maternal toxicity. No effects on the incidence of external, visceral or skeletal malformations or variations were observed. Under the conditions of this study, the NOAELs for dapsone and DGME were 30 mg/kg/day and 180 mg/kg/day, respectively. DGME did not induce toxicity under the conditions of this study.

**Study no.:** ATLS-121

**Volume #, and page #:** Mod 4, vol 60

**Conducting laboratory and location:** \_\_\_\_\_

**Date of study initiation:** 10-MAY-2000

**GLP compliance:** Yes

**QA reports:** yes (X) no ( )

**Drug, lot #, radiolabel, and % purity:** Dapsone, USP, Lot #01110C; Diethylene glycol monoethyl ether (DGME), Lot#11189A

**Formulation/vehicle:** The test materials were prepared as suspensions of dapsone or solutions of DGME. 0.5% carboxymethylcellulose in purified deionized water was used as the vehicle.

**Methods:**

Species/strain: Rabbit/New Zealand white

Doses employed: 0 (control), 6, 30, and 150mg/kg/day dapsone; a satellite group received 180mg/kg/day DGME (an excipient in the product).

Route of administration: Oral (gavage) once daily

Study design: Day on which mating was confirmed (plug or sperm in vagina) was designated day 0. Pregnant females were dosed on days 6 through 18 of gestation. Dams were killed on day 29 and C-sectioned.

Number/group: 20

Parameters and endpoints evaluated: Maternal survival and body weight.

Numbers of live, dead, and resorbed fetuses were determined. Live fetuses were weighed and examined for external, visceral, and skeletal anomalies.

**Results:**

In-life (maternal) observations:

Maternal Mortality: Two does at 150mg/kg/day delivered prematurely (day 24 and 29) and were sacrificed. No other unscheduled deaths.

Clinical signs: Increased incidence of constipation (reduced defecation) at 150mg/kg/day

Maternal body weight: Significantly reduced body weight gain throughout period of dosing at 150mg/kg/day

Food consumption: Reduced during treatment period at 150mg/kg/day

Toxicokinetics: NA

Terminal and necroscopic evaluations (offspring):

Note: The does were presumed to be pregnant (at the outset of the study), but only 18, 18, 20, 19, and 18 does were actually pregnant in the control, DGME, 6, 30, and 150 mg/kg/day groups, respectively.

Body weight of live fetuses (male/female combined; grams, mean±SD): Slightly reduced at 150 mg/kg/day

No. of Live fetuses at C-section: Significantly reduced at 150mg/kg/day only.

No. of early resorptions: Significantly increased at 150mg/kg/day only. Seven liters in 150mg/kg/day group were completely resorbed.

No. of late resorptions: No remarkable observations

Gross non-skeletal anomalies: No remarkable observations

Skeletal anomalies: No remarkable observations

Toxicokinetics: Note: TK data were not obtained in this study, but the following data were obtained in pregnant rabbits in a preliminary study that was conducted to support dosage selection for the pivotal study:

Time Point	Dose (mg/kg/day)	C <sub>max</sub> (ng/mL)	T <sub>max</sub> (hr)	AUC <sub>0-24</sub> (ng·hr/mL)
Day 6	3	443	0.5	1660
	30	5970	0.5	27,100
	100	13,500	0.5	70,200
	300	22,400	0.5	160,000
Day 18	3	569	0.5	1720
	30	8450	0.5	30,600
	100	17,600	0.5	80,200
	300	29,200	0.5	252,000

## Prenatal and postnatal development

**2.6.6.6.5 Study title:** Dapsone and diethylene glycol monoethyl ether: Oral (gavage) developmental and perinatal/postnatal reproduction toxicity study in rats, including a postnatal reproduction toxicity study in rats, including a postnatal behavioral/functional evaluation

**Key study findings:** Little toxicity was observed under the conditions of this study, although mean body weight gain was significantly reduced in high-dose F0 females over the period of gestation, indicating that a sufficiently high level of exposure was achieved for the data to be valid. The mean number of stillborn pups per litter was slightly, but statistically significantly, higher in high-dose litters than in control litters. No effects were observed on pup viability, physical development, behavior, learning ability, or reproduction.

**Study no.:** ATLS-137

**Volume #, and page #:** Mod 4, vol 63

**Conducting laboratory and location:** \_\_\_\_\_

**Date of study initiation:** 26-FEB-2001

**GLP compliance:** Yes

**QA reports:** yes (X) no ( )

**Drug, lot #, radiolabel, and % purity:** Dapsone, USP, Lot #01110D; Diethylene glycol monoethyl ether (DGME), Lot#010401-000

**Formulation/vehicle:** The test materials were prepared as suspensions of dapsone or solutions of DGME. 0.5% carboxymethylcellulose in purified deionized water was used as the vehicle.

### Methods:

Species/strain: Rat/Crl:CD(SD)IGS BR VAF/Plus

Number/sex/group: 25

Doses employed: 0 (control), 3, 12, and 30 mg/kg/day dapsone; a satellite group received 180 mg/kg/day DGME (an excipient in the product)

Route of administration: Oral (gavage) once daily,

Satellite groups used for toxicokinetics: No

Study design: F0 females were administered the test articles daily from gestation day 7 (seventh day following confirmed mating) through day 27 postpartum (or "gestation" day 24, for rats that didn't litter). F0 animals were sacrificed on day 28 postpartum. 25 F1 animals of each gender (pups of F0 animals) were selected on day 28 postpartum. At approximately 90 days of age the F1 animals were cohabitated for up to 21 days with an F1 animal of the opposite gender and from a

different litter. F1 females with a copulatory plug were considered to be at gestation day 0 and individually housed. The F1 males were sacrificed at the conclusion of the cohabitation period. F1 females were sacrificed on gestation day 21 and caesarean-sectioned.

Parameters and endpoints evaluated: Body weights, food consumption, and clinical signs of all animals were monitored. F0 females were monitored for duration of gestation, litter size, pup viability, and nursing behavior. Gross necropsies were performed. F1 animals were evaluated on approximately day 70 postpartum for performance in a water-filled M maze for overt coordination, swimming ability, learning, and memory. F1 males were monitored for the age of preputial separation and F1 females were monitored for the age of vaginal opening. F1 animals were observed for changes in mating behavior. F1 males testes and epididymides were weighed. F1 females were examined for numbers of corpora lutea, implantation sites, and viable fetuses. F2 fetuses were weighed and examined for gross external alterations.

## Results

F<sub>0</sub> in-life: One high-dose (30 mg/kg/day) F0 female was found dead during lactation. No other unscheduled deaths. All clinical signs, including the death, were considered incidental. Body weight gain was significantly reduced in high-dose F0 females over the period of gestation (gestation days 0-20). Mean body weight tended to be reduced in high-dose F0 females during the lactation period. Food consumption by F0 females tended to mirror the body weight observations. There was no effect of treatment on the duration of the gestation period, the number of rats that delivered, or the number of dams with all pups dying postpartum. The mean number of stillborn pups per litter was significantly higher with increased exposure to dapsone ( $0.0 \pm 0.2$  for control litters compared to  $0.4 \pm 1.2$  in high-dose litters,  $p < 0.01$ ). No effects were observed on pup viability postpartum.

F<sub>0</sub> necropsy: No remarkable observations.

F<sub>1</sub> physical development: No remarkable observations.

F<sub>1</sub> behavioral evaluation: No remarkable observations.

F<sub>1</sub> reproduction: No remarkable observations.

F<sub>2</sub> findings: No remarkable observations.

### 2.6.6.7 Local tolerance

**2.6.6.7.1 Study title**: Primary skin irritation study in the rabbit, study No. TA001-800, sponsor's ID number ATLS-94, in-life 6/97, study report dated 6/24/97, conducted by

\_\_\_\_\_ in compliance with Good Laboratory Practice regulations (21 CFR 58).

Six male New Zealand white rabbits were used. The back of each animal was shaved and a site in the shaved area was abraded with a needle. A second site remained unabraded. 0.5mL portions of the test article (1% dapsone gel, formula #05/44-1, lot #020-97) were placed on occlusive patches and applied to approximately 1 square inch areas of skin at both the abraded and the unabraded test sites. Following a 24 hour exposure period, the patches were removed and the application sites washed. The sites were evaluated for dermal reactions at 30 minutes and at 48 hours after removal of the test material.

**Results:** Slight erythema was observed at 1 of 6 intact test sites at both 30 minutes and at 48 hours after removal of the test material (on the same rabbit); this was probably not related to the test material (it may have been secondary to clipping). One of 6 abraded test sites exhibited slight erythema at the 30 minute time point, but not at the latter point. Again, it is likely that this erythema was not induced by the test material. No other signs of toxicity were observed.

**Conclusions:** Under the conditions of this study, the test article (1% dapsone gel) did not appear to be a primary skin irritant.

**2.6.6.7.2 Study title:** Ocular irritation study in the rabbit, study No. TA002-800, in-life 6/97, study report dated 6/26/97, conducted by \_\_\_\_\_ in compliance with Good Laboratory Practice regulations (21 CFR 58).

Six New Zealand white rabbits (gender not specified) were used. 0.1mL of the test article (1% dapsone gel, formula #05/44-1, lot #020-97) was instilled into the lower conjunctival sac of one eye of each rabbit. The opposite eye of each rabbit remained untreated as a control. The eyes were examined at 24, 48, and 72 hours after dosing, using a light source. Fluorescein and UV light were used at 24 hours.

**Results:** Several of the animals exhibited slight conjunctival redness of the treated eye at 24 and/or 48 hours after treatment; redness was not observed at 72 hours after dosing. Significant irritation of the treated eye was not judged to have occurred.

**Conclusions:** Under the conditions of this study, the test article (1% dapsone gel) did not appear to induce irritation of the ocular tissue of the rabbit.

**2.6.6.7.3 Study title:** Ocular irritation study in the rabbit (FHSA method), study No. ATLS-97, in-life 10/98, study report dated 10/28/98, conducted by \_\_\_\_\_ in compliance with Good Laboratory Practice regulations (21 CFR 58).

Six New Zealand white rabbits (gender not specified) were used. 0.1 mL of the test article (5% dapsone/25% DGME gel, lot #010-98) was instilled into the lower conjunctival sac of one eye of each rabbit. The opposite eye of each rabbit remained untreated as a control. The eyes were examined at 24, 48, and 72 hours after dosing, using a light source. Fluorescein and UV light were used at 24 hours.

**Results:** No irritation was observed in any of the animals.

**Conclusions:** Under the conditions of this study, the test article (5% dapsone gel) did not appear to induce irritation of the ocular tissue of the rabbit.

### 2.6.6.8 Special toxicology studies

**2.6.6.8.1 Study title:** Topical phototoxicity screening test of dapsone in a topical formulation in guinea pigs, study No. 522-001, sponsor's ID No. ATLS-95a, in-life 7/98, report dated 10/7/98, conducted by \_\_\_\_\_ in compliance with Good Laboratory Practice regulations (21 CFR 58).

Seven groups of 5 male Crl:(HA)BR guinea pigs were utilized. Six or eight treatment sites (approx. 2.5cm<sup>2</sup>) were located on the dorsum and were depilated 24 hours prior to application of 20µl of the assigned test material, which was applied once per site, rubbed in, and allowed to dry. The sites were not rinsed prior to irradiation. The treatment procedure is summarized below:

Group	Skin Sites	Test Material	Irradiation
1	1 and 2 3 and 4 5 and 6	10% EDG <sup>1</sup> Vehicle 1% DAP <sup>2</sup> in 10% EDG Vehicle 5% DAP in 10% EDG Vehicle	UV <sub>A</sub> /UV <sub>B</sub>
2	1 and 2 3 and 4 5 and 6	25% EDG Vehicle 1% DAP in 25% EDG Vehicle 5% DAP in 25% EDG Vehicle	UV <sub>A</sub> /UV <sub>B</sub>
3	1 and 2 3 and 4 5 and 6 7 and 8	Methanol 8-MOP <sup>3</sup> in Methanol (0.01mg/mL) 8-MOP in Methanol (0.1mg/mL) 8-MOP in Methanol (1.0mg/mL)	UV <sub>A</sub> /UV <sub>B</sub>
4	1 and 2 3 and 4 5 and 6	10% EDG Vehicle 1% DAP in 10% EDG Vehicle 5% DAP in 10% EDG Vehicle	None
5	1 and 2 3 and 4 5 and 6 7 and 8	25% EDG Vehicle 1% DAP in 25% EDG Vehicle 5% DAP in 25% EDG Vehicle	None
6	1 and 2 3 and 4 5 and 6 7 and 8	25% EDG Vehicle 1% DAP in 25% EDG Vehicle 5% DAP in 10% EDG Vehicle 5% DAP in 25% EDG Vehicle	UV <sub>A</sub>
7	1 and 2 3 and 4 5 and 6 7 and 8	Methanol 8-MOP <sup>3</sup> in Methanol (0.01mg/mL) 8-MOP in Methanol (0.1mg/mL) 8-MOP in Methanol (1.0mg/mL)	UV <sub>A</sub>

<sup>1</sup>EDG = Ethoxydiglycol

<sup>2</sup>DAP = Dapsone

<sup>3</sup>8-MOP = 8-Methoxypsoralen

UV irradiation occurred approximately 60 minutes after application of the test materials. The source for the UV<sub>A</sub>/UV<sub>B</sub> radiation (groups 1, 2, and 3) was a 6.5 Kv long arc lamp with a filter to attenuate mid-range UV radiation, and was stated to simulate mid-latitude summer sunlight. A dose of about 0.75 MED was administered over a 45 minute period. The UV<sub>A</sub> source used for groups 6 and 7 was a bank of fluorescent lamps (FR40T12/PUV<sub>A</sub>) that primarily emitted in the UV<sub>A</sub> region. A dose of approximately 10J/cm<sup>2</sup> was administered over an 80 minute period. All animals were examined for erythema once daily for 3 days after exposure.

**Results.** Phototoxicity and primary irritation were not observed in animals treated with dapsone or ethoxydiglycol, with or without irradiation. Appropriate responses were observed in positive control animals.

**Conclusions.** Under the conditions of this study, dapsone and ethoxydiglycol do not appear to induce phototoxicity or primary irritation of guinea pig skin.

**Reviewer's comments:** The absorption maximum of dapsone occurs at 291nm. Although the radiation used in this study was slanted toward the other end of the UV spectrum, I consider this study to provide meaningful data about the potential of dapsone to induce phototoxicity because: 1) light simulating sunlight was studied (the "UV<sub>A</sub>/UV<sub>B</sub>" irradiation); 2) the animals presumably were exposed to some light in the region of 291nm; and 3) the UV<sub>A</sub>/UV<sub>B</sub> light source used was similar to the light source that would be used in the proposed clinical photo study.

**2.6.6.8.2 Study title:** Topical photoallergy screening test of dapsone in a topical formulation in guinea pigs, study No. 522-002, sponsor's ID No. ATLS-95b, in-life 7/98-8/98, report dated 11/17/98, conducted by \_\_\_\_\_, in compliance with Good Laboratory Practice regulations (21 CFR 58).

**Induction phase.** An approximately 8cm<sup>2</sup> area of skin in the shoulder/dorsal region of each animal was depilated on study day zero. On day "one" the depilated areas were injected with \_\_\_\_\_ complete adjuvant and the skin was stripped with tape. Four groups of 5 male CrI:(HA)BR guinea pigs were treated with gel that contained 5% dapsone and 25% ethoxydiglycol (100μL of material per site), and two groups were topically treated with a positive control material (30mg/mL tetrachlorosalicylanilide (TCSA) in 4:1 acetone:corn oil). Approximately 30 minutes after application, two of the groups that received dapsone were irradiated with UV light, and two were not. Both of the positive control groups were irradiated. The radiation source was a bank of fluorescent lamps (FR40T12/PUV<sub>A</sub>) that apparently emitted primarily in the UV<sub>A</sub> region. A dose of approximately 10J/cm<sup>2</sup> was administered over an 80 minute period. These procedures (injection with \_\_\_\_\_ complete adjuvant, tape stripping, topical application, and irradiation) were repeated on days 2, 3, 4, and 5 of the study.

**Challenge phase.** On day 22, hair on the dorsum and sides of each animal was removed. On day 23, eight 2.5cm<sup>2</sup> depilated sites on each animal were demarcated. Animals induced with dapsone/ethoxydiglycol received duplicate applications of dapsone/ethoxydiglycol formulations in the proportions 0%/25%, 1%/10%, 1%/25%, and 5%/10%. Positive control animals were treated with TCSA. One group of animals that had been induced with dapsone and radiation, and one group that received dapsone but no radiation, were irradiated as described above, as was one of the positive control groups. The other groups were not irradiated during the challenge phase.

The animals were examined for clinical signs and dermal responses throughout the study, including daily for three days following the "challenge" applications.

**Results.** Photoallergy and contact hypersensitivity were not observed in animals treated with dapsone or ethoxydiglycol. Although slight to mild erythema was observed in some animals in the groups that received dapsone gel, the group that was irradiated during both the induction phase and the challenge phase did not exhibit a higher degree of reaction than did the other groups. Appropriate responses were observed in positive control animals.

**Conclusions.** Under the conditions of this study, dapsone and ethoxydiglycol do not appear to induce photoallergy in guinea pigs.

**Reviewer's comments:** The absorption maximum of dapsone occurs at 291nm. The radiation used in this study was apparently slanted toward the other end of the UV spectrum (UV<sub>A</sub>). Therefore, data from this study should be interpreted with caution. However, nonclinical photoallergenicity data are not regarded by the Division as being pivotal to the drug development/approval process, so additional photoallergenicity data are not required.

**2.6.6.8.3 Study title:** ISO sensitization study in the guinea pig, study No. TI260-300, sponsor's ID No. ATLS-98, in-life 10/98-11/98, study report dated 11/25/98, conducted by \_\_\_\_\_, in compliance with Good Laboratory Practice regulations (21 CFR 58).

**Induction phase.** Fifteen female Crl:(HA)BR guinea pigs were shaved on the left flank. A \_\_\_\_\_ Chamber which contained a cotton disk was secured with hypoallergenic tape to intact skin at the shaved site of each animal. Ten of the animals received disks that were saturated with 0.3mL of gel that contained 5% dapsone and 25% ethoxydiglycol (lot #010-98). The other five animals received disks that were saturated with saline (control group). After 6 to 8 hours, the test materials were removed and the sites were wiped dry. The test-article exposure procedure was repeated three times weekly for three weeks. The application sites were examined for dermal response at 24 hours after each exposure.

**Challenge phase.** At 14 days after the final induction exposure, 0.3mL of the test (or control) material was again applied to a shaved site of each animal using a pad inside a \_\_\_\_\_ Chamber. Again, the patches were removed after 6 to 8 hours and the sites wiped clean. The "challenge" site of each animal was examined for dermal reaction 24, 48, and 72 hours after removal of the patch and scored on a scale of 0 to 4 in regard to erythema and edema.

**Results.** No signs of irritation, erythema, or edema were observed on any animal at any time point during either the induction phase or the challenge phase of the study.

**Conclusions.** Under the conditions of this study, the test article (5% dapsone/25% ethoxydiglycol gel) did not appear to induce sensitization of the guinea pig.

**2.6.6.8.4 Study title:** Delayed contact sensitization study in the guinea pig, study No. TA005-300, sponsor's ID No. ATLS-93, in-life 6/97-7/97, study report dated 7/31/97, conducted by \_\_\_\_\_, in compliance with Good Laboratory Practice regulations (21 CFR 58).

Fifteen female Crl:(HA)BR guinea pigs were used. For each animal, a cotton pad inside a Chamber<sup>®</sup> was saturated with 0.3g of 1% dapsone gel (formula #05/44-1, lot #020-97) and the chamber was secured with hypoallergenic tape to intact, shaved skin on the trunk. After 6 to 8 hours, the test materials were removed and the sites wiped dry. The test-article exposure procedure was repeated three times weekly for three weeks. The application sites were examined for dermal response at 24 hours after each exposure. At 12 days after the final induction exposure, 0.3g of the test material was again applied to a shaved site of each animal (and 5 control animals that were not "induced") using a pad or patch inside a Chamber<sup>®</sup>. Again, the patches were removed after 6 to 8 hours and the sites wiped clean. The "challenge" site of each animal was examined for dermal reaction 24, 48, and 72 hours after removal of the patch and scored on a scale of 0 to 3 in regard to erythema and edema.

**Results:** No signs of irritation, erythema, or edema were observed on any animal at any time point during either the induction phase or the challenge phase of the study.

**Conclusions:** Under the conditions of this study, the test article (1% dapsone gel) did not appear to induce sensitization of the guinea pig.

#### 2.6.6.9 Discussion and Conclusions

Little toxicity was observed in nonclinical repeat-dose toxicology studies in which the product (or an enriched formulation) was topically applied to skin. No adverse effects were observed in female rats treated daily for six months, although erythrocytic parameters were slightly suppressed in male rats that received dapsone gel. No effects were observed in male or female rabbits treated topically for nine months. In rats that were orally dosed with dapsone for 90 days, treatment-related findings included cyanosis of the skin, hyperactivity, increased WBC count, decreased RBC count, hemoglobin concentration and hematocrit, increased prothrombin time, splenomegaly, mild splenic "congestion", and mild pigmentation of the spleen. Potential to induce hemolytic anemia, with splenic involvement, is a known adverse effect of ingested dapsone, but is unlikely to be associated with clinical use of dapsone topical gel due to the low systemic exposure involved. The no-adverse-effect-level (NOAEL) in the 90 day oral rat study was 3 mg/kg/day. The systemic exposures (AUC) achieved at the NOAEL in male and female rats were approximately 15 and 50 times the systemic exposure observed in patients, respectively. Dapsone was negative in an Ames assay (both with and without metabolic activation) and in a micronucleus assay. However, dapsone induced chromosomal aberrations in cultured CHO cells, suggesting that it is a clastogen. Dapsone was evaluated for carcinogenicity in a two-year oral (gavage) rat study and in a Tg.AC mouse study. Both studies were judged by the exec-CAC to be acceptable. No evidence of carcinogenicity was obtained in either study. Dapsone impaired fertility of male rats, as evidenced by a reduction in the fertility index (number of rats pregnant/number of rats mated), reduced sperm motility (percentage of observed sperm that were motile), and reduced numbers of implantations and viable embryos in the females that did become pregnant. However, an oral dose of 2 mg/kg/day had no effect on fertility parameters, and it is unlikely that clinically relevant impairment of fertility would be associated with use of the product. When administered to female rats at a dosage of 75mg/kg/day for 15

days prior to mating and for 17 days thereafter, dapsone reduced the mean number of implantations, increased the mean early resorption rate, and reduced the mean litter size. These effects were probably secondary to maternal toxicity. No effects on the incidence of external, visceral or skeletal malformations or variations were observed. Under the conditions of this study, the NOAEL for dapsone for developmental effects in rats was 12 mg/kg/day. When administered at a dosage of 150 mg/kg/day to rabbits on days 6-18 of gestation, dapsone significantly increased the incidence of early resorptions. Two does at this dosage delivered prematurely and seven does resorbed all fetuses. These effects were probably secondary to maternal toxicity. No effects on the incidence of external, visceral or skeletal malformations or variations were observed. Under the conditions of this study, the NOAEL for dapsone for developmental effects in rabbits was 30 mg/kg/day. Little toxicity was observed in a two-generation study in which F0 females were administered the test articles daily from gestation day 7 through day 27 postpartum at exposures of 3, 12, and 30 mg/kg/day dapsone or 180 mg/kg/day DGME. The mean number of stillborn pups per litter was slightly, but statistically significantly, higher in high-dose dapsone litters than in control litters. No effects were observed on pup viability, physical development, behavior, learning ability, or reproduction. Dapsone topical gel is not an irritant of skin or eyes, is not phototoxic, and is nonsensitizing.

The only excipient in the product that is of potential toxicological concern is DGME, which is present in the product at a concentration of 25% w/w. DGME was evaluated in an extensive battery of toxicology studies, including chronic topical dermal toxicology studies in rats and rabbits, a 90-day oral toxicology study in rats, a series of genetic toxicology studies, a two-year oral carcinogenicity study in rats, a series of reproductive toxicology studies, and sensitization studies. No evidence of potential to induce toxicity was obtained in those studies, even though many of the studies involved exposures that substantially exceeded the clinical level of exposure.

The clinical formulation of the drug product and the individual components of the product have been adequately evaluated for safety and the database supports the safety of the proposed use of the product.

#### 2.6.6.10 Tables and Figures

Not applicable.

#### 2.6.7 TOXICOLOGY TABULATED SUMMARY

Summary of Systemic Exposure Data at the NOAEL in Selected Nonclinical Studies:

Study Type	NOAEL*	AUC** at NOAEL (ng-hr/mL)	AUC Ratio***
90 Day Oral Rat	3 mg/kg/day	Males: 6250 Females: 20,800	Males: 18 Females: 43
6 Month Topical Rat	10% Dapsone Gel	Males: 11,294 Females: 11,102	Males: 32 Females: 23
9 Month	10% Dapsone	Males: 7671	Males: 22

Topical Rabbit	Gel	Females: 5470	Females: 11
Male fertility (Rat)	2 mg/kg/day	4469	13
Teratology (Rat)	12 mg/kg/day	83,200	173
Teratology (Rabbit)	30 mg/kg/day	30,600	64
Perinatal (Rat)	12 mg/kg/day	83,200	173

\*No-Adverse-Effect-Level; level at which no substantial toxicity was observed.

\*\*AUC = "Area under curve" when mean plasma-concentration is plotted against time.

\*\*\*AUC ratio refers to  $AUC_{\text{nonclinical}}/AUC_{\text{clinical}}$ ;  $AUC_{\text{clinical}}$  refers to the AUC values observed in patients under conditions of maximum exposure (349 ng·hr/mL in males; 481 ng·hr/mL in females). AUC ratios can be considered to be "safety factors", or margins of safety.

## OVERALL CONCLUSIONS AND RECOMMENDATIONS

Conclusions: The product is approvable with respect to nonclinical concerns.

Unresolved toxicology issues (if any): None

Recommendations: The product is approvable with respect to nonclinical concerns.

Suggested labeling:

### CARCINOGENESIS, MUTAGENESIS, IMPAIRMENT OF FERTILITY:

Dapsone was not mutagenic in a bacterial reverse mutation assay (Ames test) using *S. typhimurium* and *E. coli*, with and without metabolic activation and was negative in a micronucleus assay conducted in mice. Dapsone increased both numerical and structural aberrations in a chromosome aberration assay conducted with Chinese hamster ovary (CHO) cells.

Dapsone was not carcinogenic to rats when orally administered to females for 92 weeks or males for 100 weeks at dose levels up to 15 mg/kg/day (approximately 160 times the systemic exposure observed in human males and 300 times the systemic exposure observed in human females as a result of use of the maximum recommended topical dose, based on AUC comparisons).

No evidence of potential to induce carcinogenicity was obtained in a dermal study in which dapsone gel was topically applied to Tg.AC transgenic mice for approximately 26 weeks. Dapsone concentrations of 3%, 5%, and 10% were evaluated; 3% material was judged to be the maximum tolerated dosage.

Dapsone topical gel did not increase the rate of formation of ultra violet light-induced skin tumors when topically applied to hairless mice in a 12-month photocarcinogenicity study.

The effects of dapsone on fertility and general reproduction performance were assessed in male and female rats following oral (gavage) dosing. Dapsone reduced sperm motility at dosages of 3 mg/kg/day or greater (approximately 17 times the systemic exposure observed in human males as a result of use of the maximum recommended topical dose, based on AUC comparisons). The mean numbers of embryo implantations and viable embryos were significantly reduced in untreated females mated with males that had been dosed at 12 mg/kg/day or greater (approximately 70 times the systemic exposure observed in human males as a result of use of the maximum recommended topical dose, based on AUC comparisons), presumably due to reduced numbers or effectiveness of sperm, indicating impairment of fertility. Dapsone had no effect on male fertility at dosages of 2 mg/kg/day or less (approximately 13 times the systemic exposure observed in human males as a result of use of the maximum recommended topical dose, based on AUC comparisons). When administered to female rats at a dosage of 75mg/kg/day (approximately 800 times the systemic exposure observed in human females as a result of use of the maximum recommended topical dose, based on AUC comparisons) for 15 days prior to mating and for 17 days thereafter, dapsone reduced the mean number of implantations, increased the mean early resorption rate, and reduced the mean litter size. These effects were probably secondary to maternal toxicity.

Dapsone was assessed for effects on perinatal/postnatal pup development and postnatal maternal behavior and function in a study in which dapsone was orally administered to female rats daily beginning on the seventh day of gestation and continuing until the twenty-seventh day postpartum. Maternal toxicity (decreased body weight and food consumption) and developmental effects (increase in stillborn pups and decreased pup weight) were seen at a dapsone dose of 30 mg/kg/day (approximately 500 times the systemic exposure observed in human females as a result of use of the maximum recommended topical dose, based on AUC comparisons). No effects were observed on the viability, physical development, behavior, learning ability, or reproductive function of surviving pups.

**Pregnancy:**

**Teratogenic Effects: Pregnancy Category C.** Dapsone has been shown to have an embryocidal effect in rats and rabbits when given in doses of 75 mg/kg/day and 150 mg/kg/day (approximately 800 and 500 times the systemic exposure observed in human females as a result of use of the maximum recommended topical dose, based on AUC comparisons), respectively. These effects were probably secondary to maternal toxicity. There are no adequate and well controlled studies in pregnant women. Dapsone topical gel should be used during pregnancy only if the potential benefit justifies the potential risk to the fetus.

Reviewer: Norman A. See, Ph.D.

NDA No. 21-794

Signatures (optional):

Reviewer Signature \_\_\_\_\_

Supervisor Signature \_\_\_\_\_ Concurrence Yes \_\_\_ No \_\_\_

**APPENDIX/ATTACHMENTS**

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5/23/05 02:11:45 PM  
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Paul Brown  
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PHARMACOLOGIST

Executive CAC

Date of Meeting: April 26, 2005

Committee:

Abby Jacobs, Ph.D., HFD-024, Acting Chair  
Joseph Contrera, Ph.D., HFD-901, Member  
David Morse, Ph.D., HFD-150, Alternate Member  
Paul Brown, Ph.D., HFD-540, Supervisory Pharmacologist  
Norman See, Ph.D., HFD-540, Presenting Reviewer

Author of Draft: Norman See, Ph.D.

The following information reflects a brief summary of the Committee discussion and its recommendations. Detailed study information can be found in the individual review.

NDA 21-794

Drug Name: Dapsone

Sponsor: Atrix Laboratories, Inc.

**104-Week Carcinogenicity Study in Rats**

Background: The sponsor conducted a study in which rats were orally dosed with dapsone at exposures of either 0, 1, 5, or 15 mg/kg/day. A satellite group of animals received 540 mg/kg/day of DGME by gavage. Due to poor survival in the control group, it was necessary to terminate the study prematurely (surviving females were terminated during treatment week 92; surviving males during treatment week 100). Two equivocal differences in tumor incidence were observed:

1. A trend test suggested the incidence of hemangiosarcomas in male animals increased with increasing exposure to dapsone, but a pair-wise comparison of the control and high-dose groups was negative ( $p \leq 0.0750$ ).
2. The incidence of skin papillomas in male animals appeared in a trend test to be related to exposure to dapsone, but a pair-wise comparison of the control and high-dose groups was negative ( $p \leq 0.0625$ ).

Executive CAC Recommendations and Conclusions concerning the oral rat bioassay:

The committee found that the study was minimally adequate but acceptable. The committee decided there were no clearly test-article-related neoplasms, including hemangiosarcomas and skin papillomas.

### **Dermal Carcinogenicity Study in Tg.AC Mice**

Background: The sponsor conducted a study in which Tg.AC mice were topically dosed with either the vehicle for dapsone topical gel (which contains 25% DGME), vehicle that contained either 3%, 5%, or 10% dapsone, or a control material. Topically applied dapsone did not appear to impact the formation of papillomas in Tg.AC mice under the conditions of this study. None of the mid-dose females or high-dose males or females survived to scheduled sacrifice.

Executive CAC Recommendations and Conclusions concerning the study in Tg.AC mice:

The committee concluded that this was a valid carcinogenicity assay and that no evidence of carcinogenicity had been detected.

Abigail Jacobs, Ph.D.  
Acting Chair, Executive CAC

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Abby Jacobs

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