

A summary of the toxicokinetic parameters (mean  $\pm$  SD) for male animals is provided in the following table.

Dose (mg/kg/day)	C <sub>max</sub> (ng/ml)	T <sub>max</sub> (hr)	AUC <sub>0-6 hr</sub> (ng·hr/ml)	AUC <sub>0-24 hr</sub> (ng·hr/ml)
2	5.0 $\pm$ 5.1	0.70 $\pm$ 0.76	12.1 $\pm$ 9.0	33.6 $\pm$ 15.3
10	33.3 $\pm$ 24.4	0.50 $\pm$ 0.0	83.9 $\pm$ 58.1	203 $\pm$ 82
45	168 $\pm$ 98	0.50 $\pm$ 0.0	447 $\pm$ 247	872 $\pm$ 330

A dose dependent increase in ASM 981 systemic exposure in males was noted in this study. The AUC<sub>0-6 hr</sub> levels measured in males were comparable to the AUC<sub>0-6 hr</sub> levels measured in pregnant females.

#### Male Fertility and Reproductive

##### Performance:

No treatment related effects on sperm motility or testicular sperm head count was noted in this study. No treatment related effects on male mating index or male fertility index were noted in this study.

#### Female Fertility and Reproductive

##### Performance:

No treatment related effects on female mating index, female pregnancy rate, mean numbers of corpora lutea, mean numbers of implantation sites or preimplantation sites was noted in this study. The percent post implantation loss was significantly greater in high dose animals (36.0%) compared to control animals (8.2%). The mean number of live fetuses was significantly reduced in high dose animals (7.25) compared to control animals (9.11). The sex ratio of male to female fetuses was not affected in any treatment group.

#### Fetal Parameters:

No treatment related effect on placental weight was noted in this study. Mean fetal weight was decreased in the high dose group ( $\downarrow$ 8.8%) compared to the control group. No treatment related external findings were noted in this study. No treatment related soft tissue or skeletal malformations were noted in this study.

The percentage of fetuses or litters with skeletal retardation (effect on ossification) was increased in the high dose group (24% fetal and 81% litter incidence) compared to the control group (5.2% fetal and 42% litter incidence). The retardation was classified as an increase in unossified cervical vertebral bodies and retarded ossifications of thoracic vertebral bodies. The effects on ossification correlated with the decrease in fetal

weight. Delayed ossification is noted as a toxic effect, not as a malformation.

### Summary of individual study findings:

No treatment related effects on paternal toxicity or male reproductive performance were noted in this study. It may have been possible to test a higher dose in males in this study due to the lack of toxicity observed at the high dose. The NOAEL for paternal toxicity, male fertility and reproductive performance is 45 mg/kg/day ( $AUC_{0-24 \text{ hr}} = 872 \text{ ng}\cdot\text{hr}/\text{ml}$ ), which was the highest dose tested in this study. Female fertility and reproductive performance were affected in the absence of maternal toxicity in high dose animals. Embryotoxicity and estrus cycle disruption were noted in high dose females (45 mg/kg/day;  $AUC_{0-24 \text{ hr}} = 1448 \text{ ng}\cdot\text{hr}/\text{ml}$ ). The NOAEL for female fertility and reproductive performance is 10 mg/kg/day ( $AUC_{0-24 \text{ hr}} = 465 \text{ ng}\cdot\text{hr}/\text{ml}$ ).

Reduced fetal weights were noted in the high dose group. No malformations were noted in this study. An increased incidence in fetal retardation (delayed ossification) was noted in the high dose group. This was attributed to the embryofetal toxicity that was noted in the high dose group. An increase in embryofetal toxicity and postimplantation loss were noted in high dose females. This may be interpreted as a potential signal for teratogenicity in this study. The NOAEL for malformations was 45 mg/kg/day ( $AUC_{0-24 \text{ hr}} = 1448 \text{ ng}\cdot\text{hr}/\text{ml}$ ).

### Reproductive Toxicology Study #3:

*An oral pre- and post-natal development in rats (Drug form: lyophilisate suspension)*

**Study title:** An oral pre- and post-natal development in rats (Drug form: lyophilisate suspension)

**Study no.:** T-113/203-160

**Lab Study No.:** 96/SPM088/1035

**Volume #, and page #:** 70, 5-1

**Conducting laboratory:** \_\_\_\_\_

**Date of study initiation:** 1/29/96

**GLP compliance:** Yes

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

**Drug, and lot#:** ASM 981 – batch# Y294 1194

**Formulation/vehicle:** Water & Plasmagelan<sup>R</sup> (Note: ASM 981 lyophilisate was reconstituted using water and then further diluted with the plasma volume surrogate Plasmagelan<sup>R</sup> to adjust to the final drug concentrations)

**Methods:**

**Species/strain:** Female Wistar rats; 11 – 12 weeks old; 205 – 247 grams  
**Doses employed:** 0, 2, 10 and 40 mg/kg/day  
**Route of administration:** Oral (gavage); Dose volumes = 4.0, 1.0 (diluted solution), 1.0 and 4.0 ml/kg for doses of 0, 2, 10, and 40 mg/kg/day

**Study design:**

Test article or vehicle (Plasmagelan<sup>R</sup>) was administered orally (via gavage) on a daily basis for 7 days/week. Female rats were treated from gestation day 6 until lactation day 21. Females rats were mated with control untreated male animals that were not part of the study. F<sub>0</sub> females gave birth naturally and reared their offspring to lactation day 21 (day of weaning). F<sub>0</sub> females were sacrificed on lactation day 21. Representative numbers of F<sub>1</sub> offspring were reared to maturity and allowed to mate to determine the reproductive potential of the F<sub>1</sub> generation.

**Number/sex/group:** 22 females/dose (F<sub>0</sub> generation); 20/sex/dose (F<sub>1</sub> generation; Note – no offspring were available from the high dose group for evaluation)

**Parameters and endpoints evaluated:**

In life toxicity parameters evaluated for F<sub>0</sub> females in this study included mortality (daily), clinical signs (daily), body weights (daily) and food consumption (daily). In addition, individual gestation length (from the day of mating until parturition commenced) was calculated for F<sub>0</sub> females.

Terminal evaluations in F<sub>0</sub> females included a gross necropsy conducted following weaning or total litter death. The number of implantation sites was recorded for each F<sub>0</sub> female. Any abnormal tissues were preserved for possible future histopathological evaluation. Terminal evaluations in F<sub>1</sub> offspring that were not selected for further study was a gross necropsy conducted at 8 weeks of age. Any abnormal tissues were preserved for possible future histopathological evaluation.

The following in life parameters were evaluated for the litters of F<sub>0</sub> females. The following was recorded for each liter 24 hours after birth: number born (live or dead), individual bodyweights, individual sexes and general overall observations of the offspring. In life toxicity parameters for F<sub>1</sub> offspring included mortality (daily), clinical signs (daily), litter size (daily), body weight (days 1, 4, 7, 11, 14, 18, 21 and 28 days of age). F<sub>1</sub> offspring were culled on day 4 to 4 male and 4 females per liter. Physical development was assessed by noting the day of onset and completion of the following parameters: Pinna unfolding, hair growth, tooth eruption, eye opening, vaginal opening and balano-preputial separation. Auditory (startle response to noise) and visual responses (pupil closure response or dark adapted eyes to bright light and visual placing response) of F<sub>1</sub> offspring were measured on day 25. The following developmental parameters were assessed in F<sub>1</sub> offspring. Activity level was determined on day 26 by monitoring in cage activity over a 24 hour period via infra-red light sources and detectors. A water maze test was administered on day 27 as a measure of learning behavior. Nueuromuscular

function was assessed during day 28 – 30 with the following tests: traversing flat and round rods, rotarod treadmill, mid-air righting reflex, fore- and hind-limb wire hanging and grid-gripping ability.

At ~5 weeks of age, following completion of the behavioral and neuromuscular function tests, 20 male and 20 female were selected at random for the remainder of the study. These animals were used for assessment of physical and sexual maturation and reproductive performance of progeny from test article treated animals. It is important to note that due to the high embryoletality in the high dose group, no offspring were available from the high dose group for evaluation in the next phase of this study.

In life toxicity parameters evaluated for F<sub>1</sub> animals included clinical signs (daily) and body weights (males – weekly until termination; females weekly until mating and then on days 0, 3, 7, 10 and 14 during gestation). F<sub>1</sub> males and females were paired for mating at ~10 – 11 weeks of age.

Terminal evaluations in F<sub>1</sub> animals included the following. F<sub>1</sub> females were sacrificed on day 14 after mating for a gross necropsy and any abnormal tissues were preserved for possible future histopathological evaluation. The number of corpora leutea, number of implantation sites, number of resorption sites and number and distribution of fetuses (live and dead) were recorded for each F<sub>1</sub> female. F<sub>1</sub> males were sacrificed after the gross necropsy of the F<sub>1</sub> females. A gross necropsy was performed on F<sub>1</sub> males and any abnormal tissues were preserved for possible future histopathological evaluation.

### Results:

#### In life toxicity parameters – F<sub>0</sub> females

- Mortality:** No treatment related effects on mortality were noted in this study.
- Clinical signs:** Mid and high dose F<sub>0</sub> females had an increased incidence of small, round and hard feces noted during the gestation and lactation periods. Twenty high dose females failed to litter. The remaining two animals in the high dose group gave birth. However, it was decided that data from only two animals in the high dose group would be of limited value. Therefore, data on fetuses from the high dose was not obtained in this study.
- Body weight:** Body weight was slightly decreased in mid dose F<sub>0</sub> females (↓5.6%) by the end of the gestation period. Body weight was significantly decreased in high dose F<sub>0</sub> females by the end of the gestation period due to loss of litters. To determine an accurate body weight for the mid and high dose F<sub>0</sub> females it would need to be corrected for the uterine weight. This information was not provided in the study report. The extensive weight loss noted in high dose F<sub>0</sub> females was probably related to loss of the litter as opposed to a direct toxicological effect.

No treatment related effect on body weight in F<sub>0</sub> females was noted during the lactation period.

**Food consumption:** A decrease in food consumption was noted in mid dose F<sub>0</sub> females during the gestation period. No treatment related effects on food consumption in F<sub>0</sub> females were noted during the lactation period.

**Gestation length:** No treatment related effect on gestation length was noted in this study.

#### Terminal Evaluations – F<sub>0</sub> females

**Gross Pathology:** No treatment related macroscopic findings were noted in F<sub>0</sub> females. No treatment related macroscopic findings were noted in the high dose F<sub>1</sub> fetuses that died before termination. No treatment related effects on the number of implantation sites was noted in low and mid dose F<sub>0</sub> females.

#### In life parameters for litters of F<sub>0</sub> females

**Litter size:** Post-implantation survival was very low in the high dose group. Twenty high dose F<sub>0</sub> females failed to litter. Of the two remaining high dose females, one gave birth to only five offspring from 11 implantations. Survival was unaffected in the other high dose F<sub>0</sub> female. The number of implantation sites was slightly lower in the mid dose F<sub>0</sub> females (↓10.4%) compared to control F<sub>0</sub> females. The number of offspring at day 1 was lower in mid dose F<sub>0</sub> females (↓15.7%) compared to control F<sub>0</sub> females. No treatment related effects on litter size or survival were noted in low dose F<sub>0</sub> females.

**Sex ratio:** No treatment related effects on sex ratio of F<sub>1</sub> fetuses from low and mid dose F<sub>0</sub> females were noted in this study.

**Bodyweights:** No treatment related effects on bodyweight of F<sub>1</sub> fetuses from low and mid dose F<sub>0</sub> females was noted in this study.

**Physical Development:** No treatment related effects on pinna unfolding, hair growth, tooth eruption, eye opening and the completion of vaginal opening and preputial separation were noted in F<sub>1</sub> fetuses from low and mid dose F<sub>0</sub> females.

#### **Auditory and Visual**

**Response:** No treatment related effects on auditory or visual responses were noted in F<sub>1</sub> fetuses from low and mid dose F<sub>0</sub> females.

**Developmental**

**Parameters:** No treatment related effects on locomotor activity, water maze performance or neuromuscular function were noted in F<sub>1</sub> fetuses from low and mid dose F<sub>0</sub> females.

**In life toxicity parameters – F<sub>1</sub> animals**

**Clinical Signs:** No treatment related effects on clinical signs were noted in F<sub>1</sub> animals.

**Body weights:** No treatment related effects on body weights were noted in F<sub>1</sub> animals.

**Mating Performance**

**and Fertility:** No treatment related effects on mating performance or fertility were noted in F<sub>1</sub> animals.

**Terminal Evaluations – F<sub>1</sub> animals****Male Gross**

**Pathology:** No treatment related macroscopic findings were noted in F<sub>1</sub> males.

**Female Gross**

**Pathology:** No treatment related macroscopic findings were noted in F<sub>1</sub> females.

**Litter Effects:** No treatment related effects on the number of corpora leutea, number of implantation sites, number of resorption sites and number and distribution of fetuses (live and dead) were noted in F<sub>1</sub> females.

**Summary of individual study findings:**

Marked embryotoxicity was noted in the high dose group. Only two out of 22 females gave birth in the high dose group. The high dose group was not evaluated in the remainder of the study due to the high level of embryotoxicity. Some indication of embryotoxicity was noted in the mid dose group as fewer offspring and a low post-implantation survival. The NOAEL for embryo-fetal toxicity is 2 mg/kg/day. Post natal survival, development of the F<sub>1</sub> generation and their subsequent maturation and fertility was not affected in the low and mid dose groups. The NOAEL for post-natal toxicity is 10 mg/kg/day.

**Reproductive Toxicology Study #4:**

*A dermal embryo-fetal development study in rats (Drug form: MF cream)*

**Study title:** A dermal embryo-fetal development study in rats (Drug form: MF cream)

**Key study findings:** No signal for teratogenicity was noted in this study. No effects on maternal toxicity or female reproductive performance were noted in this study.

Study no.: T-114/203-210  
Study No.: 6135-156  
Volume #, and page #: 71, 5-1  
Conducting laboratory: \_\_\_\_\_  
Date of study initiation: 11/17/97  
GLP compliance: Yes  
QA- Report: Yes (X) No ()  
Drug, and lot#: 0.2% ASM 981 cream – batch# Z008 0297  
0.6% ASM 981 cream – batch# Z010 0297  
1.0% ASM 981 cream – batch# Z004 1097  
Formulation/vehicle: Vehicle cream – batch# Z068 0896

Methods:

**Species/strain:** Mated female Wistar rats; 11 weeks old; 178 – 225 grams  
**Doses employed:** 0, 2 (0.2%), 6 (0.6%) and 10 (1.0%) mg/kg/day  
**Route of administration:** Topical; Dose volume = 1 gm/kg/day  
**Study design:**

Prior to treatment, the hair was clipped from an area 2 inches down the back starting at the nape of the neck and was 2 inches side. Clipping was repeated on an as needed basis. Test article was applied to the treatment area, rubbed in with the use of a finger cot, covered with gauze and secured with a harness tubing that was fitted around the animals thoracic region. The harness and gauze were removed after 6 hours of treatment and the dose site was wiped a clean gauze to remove any residual test article. Animals were treated from gestation days 6 – 21.

**Number/sex/group:** 20 females/dose in main study; 5 females/dose in TK satellite group

**Parameters and endpoints evaluated:**

Toxicity parameters evaluated in this study included mortality (daily), clinical signs (daily), body weights (gestation days 0, 4, 6, 8, 10, 12, 14, 16, 18 and 21), food consumption (gestation days 4, 6, 8, 10, 12, 14, 16, 18 and 21) and gross necropsy. The gross necropsy was conducted for main study females only on gestational day 21.

The following parameters were measured during the gross necropsy in females: the number of implantation sites, the number of early and late resorptions, live and dead fetuses, number of implantation sites and number of corpora lutea. All fetuses were examined for external findings. Examination of the viable F1 fetuses included body weight and fetal sex. One half of the fetuses were examined for soft tissue abnormalities and the other half of the fetuses were examined for skeletal abnormalities.

Blood samples were obtained from satellite female rats on gestation day 17 on 0, 2, 4 and 6 hours after application of the cream. The live embryos were pooled per litter from satellite

female rats for analysis of test article concentration after the last blood collection point. ASM 981 levels in blood samples and fetal tissue samples were determined by \_\_\_\_\_

quantification was \_\_\_\_\_mg/ml for blood samples and \_\_\_\_\_ng/g for embryonic tissue.

#### Results:

- Mortality:** No treatment related effects on mortality were noted in this study.
- Clinical signs:** No treatment related effects on clinical signs were noted in this study.
- Body weight:** No treatment related effects on body weight were noted in this study.
- Food consumption:** No treatment related effects on food consumption were noted in this study.
- Gross Pathology:** No treatment related macroscopic findings were noted in this study.
- Toxicokinetics:** Only a few animals had detectable levels of test article in blood in this study. AUC values could not be calculated in this study. The mean  $C_{max}$  for low, mid and high dose animals was 0.2, 1.2 and 2.6 ng/ml, respectively. Highest blood levels were noted at 4 hours post dose. Detectable ASM 981 levels were only found in the mid and high dose group. The embryo levels (mean  $\pm$  SD) in the mid and high dose groups were  $0.2 \pm 0.2$  and  $0.3 \pm 0.3$  ng/g, respectively.
- Female Reproductive Performance:** No treatment related effects on female pregnancy rate, the number of implantation sites, the number of early and late resorptions, live and dead fetuses, number of implantation sites and number of corpora lutea were noted in this study. The sex ratio of male to female fetuses was not affected in any treatment group.
- Fetal Parameters:** No treatment related effect on fetal body weight was noted in this study. No treatment related external findings were noted in this study. No treatment related soft tissue or skeletal malformations were noted in this study.

#### **Summary of individual study findings:**

No treatment related effects on maternal toxicity or female reproductive performance were noted in this study. The topical NOAEL for maternal toxicity and female reproductive performance is 10 mg/kg/day (1% ASM 981 cream). No signal of teratogenicity was noted in this study. The topical NOAEL for teratogenicity was 10 mg/kg/day (1% ASM 981 cream).

Very low levels of systemic exposure were noted in this dermal teratogenicity study in Wistar rats. This explains the negative effects noted in this study. It is important to note that it would have been preferable if the daily treatment duration was for 24 hours instead of for 6 hours.

#### **Reproductive Toxicology Study #5:**

*Oral embryo-fetal development dose-range finding study in rabbits with toxicokinetic and placental transfer (Drug form: solid dispersion)*

**Study title:** Oral embryo-fetal development dose-range finding study in rabbits with toxicokinetic and placental transfer (Drug form: solid dispersion)

**Key study findings:** The results from this study suggested that the doses of 0, 2, 6 and 20 mg/kg/day ASM 981 would be adequate for the definitive oral embryo-fetal development studies in rabbits.

**Study No.:** T-115/203-096  
**Sandoz Study No.:** 2055K  
**Volume #, and page #:** 72, 5-1  
**Conducting laboratory:** Sandoz Pharma Ltd., Basle, Switzerland  
**Date of study initiation:** 3/22/95  
**GLP compliance:** Yes  
**QA- Report:** Yes (X) No ()  
**Drug, and lot#:** ASM 981 – batch# X021 0395  
**Formulation/vehicle:** Aqueous solution containing 0.5% Poloxamer 188 and 3.5% hydroxypropylmethyl cellulose (Note: The stock oral ASM 981 solution was referred to as the solid dispersion and consisted of 20% ASM 981 (w/w) solid dispersion in 0.5% Poloxamer 188 and 3.5% hydroxypropylmethyl cellulose as a carrier in water.)

#### **Methods:**

**Species/strain:** Female pregnant New Zealand rabbits; 19 – 21 weeks old; ~3 kg  
**Doses employed:** 0, 22, 30, 45, 60 and 90 mg/kg/day  
**Route of administration:** Oral (gavage); Dose volumes = 3, 1.1, 1.5, 2.25, 3.0 and 4.5 ml/kg for doses of 0, 22, 30, 45, 60 and 90 mg/kg/day

#### **Study design:**

Test article or vehicle (aqueous solution containing 0.5% Poloxamer 188 and 3.5% hydroxypropylmethyl cellulose) was administered orally (via gavage) on a daily basis from gestational day 6 – 18. Pregnant female rabbits were scheduled for sacrifice on gestational day 18.

**Number/sex/group:** 5 females/dose

**Parameters and endpoints evaluated:**

Toxicity parameters evaluated in this study included mortality (daily), clinical signs (daily), body weights (daily), food consumption (daily) and gross necropsy (on gestational day 18). The following parameters were measured during the gross necropsy: the number of resorptions, live and dead fetuses, number of implantation sites and number of corpora lutea. In addition, all fetuses were examined for external findings.

Blood samples were obtained on the last day of treatment from all treated animals at 0, 0.5, 1, 2, 4 and 6 hours after the last drug administration. Live embryos were pooled per litter after the last blood sampling. ASM 981 levels in blood samples and fetal tissue samples were determined by \_\_\_\_\_

\_\_\_\_\_ The limit of quantification was \_\_\_\_\_ ng/ml for blood and \_\_\_\_\_ ng/g for embryonic tissue.

The following reproductive parameters were determined in this study: female pregnancy rate (%), preimplantation loss (%) and postimplantation loss (%).

**Results:**

- Mortality:** No treatment related effects on mortality were noted in this study.
- Clinical signs:** No treatment related effects on clinical signs were noted in this study.
- Body weight:** Decreased body weight was noted in all ASM 981 treatment groups (22 mg/kg/day = ↓9.7%, 30 mg/kg/day = ↓5.3%, 45 mg/kg/day = ↓8.5%, 60 mg/kg/day = ↓6.0% and 90 mg/kg/day = ↓4.8%) compared to control animals.
- Food consumption:** Food consumption was decreased in the 45 (↓33.4%), 60 (↓18.0%) and 90 mg/kg/day (↓37.1%) dose groups compared to control animals.
- Gross Pathology:** No treatment related macroscopic findings were noted for the female animals in this study. In addition, no treatment related external fetal malformations were noted in this study.
- Toxicokinetics:** A summary of the toxicokinetic parameters (mean ± SD) is provided in the following table.

**APPEARS THIS WAY  
ON ORIGINAL**

Dose (mg/kg/day)	C <sub>max</sub> (ng/ml)	T <sub>max</sub> (hr)	AUC <sub>0-6 hr</sub> (ng·hr/ml)	Embryo Conc. (ng/g)	Embryo/ Plasma <sub>6 hr</sub> ratio
22	25.9 ± 8.0	1.0 ± 0.0	96 ± 11	12.4 ± 1.2	1.0 ± 0.1
30	33.2 ± 11.6	0.5 ± 0.0	139 ± 49	25.5 ± 9.5	1.3 ± 0.1
45	52.5 ± 25.9	0.6 ± 0.2	201 ± 82	36.5 ± 26.8	1.2 ± 0.3
60	84.2 ± 6.6	0.8 ± 0.4	272 ± 25	42.4 ± 8.5	1.3 ± 0.0
90	129 ± 28	0.6 ± 0.4	344 ± 27	44.8 ± 23.3	1.2 ± 0.4

An approximate dose dependent increase in ASM 981 systemic exposure was noted over the dose range tested in this study.

The embryo concentrations of ASM 981 increased ~3X from the 22 to 45 mg/kg/day dose level. The embryo concentrations of ASM 981 increased 1.2X from the 45 to 90 mg/kg/day dose level indicating a potential plateau effect at the two highest dose levels. The concentration ratios for embryonic tissue/maternal plasma at 6 hr ranged from 1.0 – 1.3 over the dose range tested. This ratio indicated good placental transfer of ASM 981 to the fetal compartment.

**Reproduction Data:** No treatment related effects on the female pregnancy index, number of corpora lutea and pre and post implantation losses were noted in this study. The mean number of implantation sites (↓12.2%) and live fetuses (↓15.8%) were slightly reduced in the 90 mg/kg/day dose group compared to control animals. It is stated in the final study report that this result from one female having only one implantation site.

#### Summary of individual study findings:

A treatment related decrease in body weight was noted for all dose groups in this study (ranged from 4.8% - 9.7%). It would have been beneficial if the body weights were corrected for uterine weights. However, this information was not provided in the study report. A decrease in food consumption was noted in the 45 (↓33.4%), 60 (↓18.0%) and 90 mg/kg/day (↓37.1%) dose groups. Possible effects on some reproductive parameters (decreased number of implantation sites and live fetuses) was noted in the 90 mg/kg/day dose group. Since slight general maternal toxicity (decreased body weight) was noted in all dose groups, a NOAEL for maternal toxicity could not be established in this study. Therefore, the study report states that the main embryotoxicity study should be conducted with doses of 0, 2, 6 and 20 mg/kg/day ASM 981. The extent of maternal toxicity noted in this study was slight at best. It would have been preferable to have included a higher dose that would elicit some frank maternal toxicity in the definitive study. However, the study was completed without any agency guidance.

APPEARS THIS WAY  
ON ORIGINAL

**Reproductive Toxicology Study 6:**

*An oral embryo-fetal development study in rabbits (Drug form: solid dispersion)*

**Study title:** An oral embryo-fetal development study in rabbits (Drug form: solid dispersion)

**Key study findings:** No signal of teratogenicity was noted in this study. No effects on female reproductive performance were noted in this study.

**Study No.:** T-116/203-140  
**Sandoz Study No.:** 4057K  
**Volume #, and page #:** 72, 5-134  
**Conducting laboratory:** Sandoz Pharma Ltd., Basle, Switzerland  
**Date of study initiation:** 6/4/95  
**GLP compliance:** Yes  
**QA- Report:** Yes (X) No ()  
**Drug, and lot#:** ASM 981 – batch# X054 0495  
**Formulation/vehicle:** Aqueous solution containing 0.5% Poloxamer 188 and 3.5% hydroxypropylmethyl cellulose (Note: The stock oral ASM 981 solution was referred to as the solid dispersion and consisted of 20% ASM 981 (w/w) solid dispersion in 0.5% Poloxamer 188 and 3.5% hydroxypropylmethyl cellulose as a carrier in water.)

**Methods:**

**Species/strain:** Female pregnant New Zealand rabbits; 18 – 24 weeks old; ~4 kg  
**Doses employed:** 0, 2, 6 and 20 mg/kg/day  
**Route of administration:** Oral (gavage); Dose volumes = 1.0, 0.1, 0.3 and 1.0 ml/kg for doses of 0, 2, 6 and 20 mg/kg/day

**Study design:**

Test article or vehicle (aqueous solution containing 0.5% Poloxamer 188 and 3.5% hydroxypropylmethyl cellulose) was administered orally (via gavage) on a daily basis from gestational day 6 – 18. Pregnant main study female rabbits were scheduled for sacrifice on gestational day 29 and TK satellite female rabbits were scheduled for sacrifice on gestational day 18.

**Number/sex/group:** 20 females/dose in the main study; 5 females/dose in TK satellite group

**Parameters and endpoints evaluated:**

Toxicity parameters evaluated in this study included mortality (daily), clinical signs (daily), body weights (daily), food consumption (daily) and gross necropsy (on gestational day 29). The following parameters were measured during the gross necropsy: the number of resorptions, live and dead fetuses, number of implantation sites and number of corpora lutea. In

addition, all fetuses were examined for external findings. Examination of the viable F1 fetuses included body weight, placental weights and fetal sex. All fetuses were examined for soft tissue and skeletal abnormalities.

Blood samples were obtained on the last day of treatment from all treated animals at 0, 0.5, 1, 2, 4 and 6 hours after the last drug administration. Live embryos were pooled per litter after the last blood sampling. ASM 981 levels in blood samples and fetal tissue samples were determined by \_\_\_\_\_

\_\_\_\_\_ The limit of quantification was  $\text{-- ng/ml}$  for blood and  $\text{-- } \mu\text{g/g}$  for embryonic tissue.

The following reproductive parameters were determined in this study: female pregnancy rate (%), preimplantation loss (%) and postimplantation loss (%).

#### Results:

- Mortality:** No treatment related effects on mortality were noted in this study.
- Clinical signs:** No treatment related effects on clinical signs were noted in this study.
- Body weight:** Slightly decreased body weight was noted in high dose females ( $\downarrow 2.5\%$ ) compared to control animals at the end of the treatment period. This slight decrease in body weight is not toxicologically significant. Body weight returned to control levels at the time necropsy (day 29).
- Food consumption:** Food consumption was marginally decreased in the high dose females compared to control females during the treatment period.
- Gross Pathology:** No treatment related macroscopic findings were noted for the female animals in this study. In addition, no treatment related external fetal malformations were noted in this study.
- Toxicokinetics:** A summary of the toxicokinetic parameters (mean  $\pm$  SD) is provided in the following table.

Dose (mg/kg/day)	$C_{\max}$ (ng/ml)	$T_{\max}$ (hr)	$AUC_{0-6 \text{ hr}}$ (ng·hr/ml)	Embryo Conc. (ng/g)	Embryo/ Plasma <sub>6 hr</sub> ratio
2	$4.1 \pm 0.2$	$0.5 \pm 0.0$	$15.0 \pm 3.3$	$0.7 \pm 0.8$	$0.4 \pm 0.4$
6	$9.1 \pm 2.6$	$1.5 \pm 1.6$	$39.3 \pm 9.7$	$3.2 \pm 0.9$	$0.6 \pm 0.1$
20	$20.9 \pm 12.3$	$1.3 \pm 1.5$	$73.6 \pm 25.1$	$10.0 \pm 2.3$	$1.1 \pm 0.3$

Note:  $AUC_{0-6 \text{ hr}}$  values were the only values provided in the study report. However, extrapolated  $AUC_{0-24 \text{ hr}}$  values for the 20 mg/kg/day dose group was provided in the label section of the NDA for purposes of calculating multiples of human exposure. The extrapolated  $AUC_{0-24 \text{ hr}}$  values for the 20 mg/kg/day dose group was 147 ng·hr/ml.

An approximate dose dependent increase in ASM 981 systemic exposure was noted over the dose range tested in this study.

The embryo concentrations of ASM 981 increased ~15X over the dose range. The concentration ratios for embryonic tissue/maternal plasma at 6 hr ranged from 0.4 – 1.1 over the dose range tested. This ratio indicated good placental transfer of ASM 981 to the fetal compartment.

**Reproduction Data:** No treatment related effects on the female pregnancy index, the mean number of implantation sites, mean number of corpora lutea and pre and post implantation losses were noted in this study.

**Fetal Parameters:** No treatment related effects on placental or fetal weight were noted in this study. No treatment related effects on the mean number of live fetuses per litter and the sex distribution were noted in this study. No treatment related external findings were noted in this study. No treatment related soft tissue or skeletal malformations were noted in this study.

#### **Summary of individual study findings:**

No toxicologically relevant treatment related effects were noted in this study. The NOAEL for maternal toxicity is 20 mg/kg/day ( $AUC_{0-6 \text{ hr}} = 74 \text{ ng}\cdot\text{hr}/\text{ml}$ ;  $AUC_{0-24 \text{ hr}} = 147 \text{ ng}\cdot\text{hr}/\text{ml}$ ), which was the highest dose tested in this study.

No treatment related effects on reproductive parameters were noted in this study. No treatment related skeletal or soft tissue malformations were noted in this study. The NOAEL for teratogenicity was 20 mg/kg/day ( $AUC_{0-6 \text{ hr}} = 74 \text{ ng}\cdot\text{hr}/\text{ml}$ ;  $AUC_{0-24 \text{ hr}} = 147 \text{ ng}\cdot\text{hr}/\text{ml}$ ), which was the highest dose tested in this study.

The dose range used in this study may not have been adequate. It would have been preferable to have used a high dose that would have expressed frank toxicity. Therefore, the doses used in this study may not have been high enough to determine a potentially teratogenic dose for ASM 981 in rabbits after oral administration.

#### **Reproductive Toxicology Study #7:**

*A dermal embryo-fetal development study in rabbits (Drug form: MF cream)*

**Study title:** A dermal embryo-fetal development study in rabbits (Drug form: MF cream)

**Key study findings:** No signal for teratogenicity was noted in this study. No effects on maternal toxicity or female reproductive performance were noted in this study.

**Study no.:** T-117/203-203  
**Study No.:** 6135-157

Volume #, and page #: 73, 5-1  
Conducting laboratory: \_\_\_\_\_  
Date of study initiation: 11/22/97  
GLP compliance: Yes  
QA- Report: Yes (X) No ()  
Drug, and lot#: 0.2% ASM 981 cream – batch# Z008 0297  
0.6% ASM 981 cream – batch# Z010 0297  
1.0% ASM 981 cream – batch# Z004 1097  
Formulation/vehicle: Vehicle cream – batch# Z068 0896

Methods:

**Species/strain:** Mated female New Zealand rabbits; 5½ months old; 3003 – 4223 grams  
**Doses employed:** 0, 2 (0.2%), 6 (0.6%) and 10 (1.0%) mg/kg/day  
**Route of administration:** Topical; Dose volume = 1 gm/kg/day  
**Study design:**

Prior to treatment, the hair was clipped from an area 3 inches down the back starting at the nape of the neck and was 3 inches on the side. Clipping was repeated on an as needed basis. Test article was applied to the treatment area, covered with gauze and secured with a harness tubing that was fitted around the animals thoracic region. An Elizabethan collar was also fitted to each animal during the treatment period. The harness and gauze were removed after 6 hours of treatment and the dose site was wiped a clean gauze to remove any residual test article. Animals were treated from gestation days 7 – 20.

**Number/sex/group:** 20 females/dose in main study; 5 females/dose in TK satellite group

**Parameters and endpoints evaluated:**

Toxicity parameters evaluated in this study included mortality (daily), clinical signs (daily), body weights (gestation days 0, 4, 7, 9, 11, 15, 18, 21 and 29), food consumption (gestation days 0, 4, 7, 9, 11, 15, 18, 21 and 29) and gross necropsy. The gross necropsy was conducted for main study females only on gestational day 29.

The following parameters were measured during the gross necropsy in females: the number of implantation sites, the number of early and late resorptions, live and dead fetuses, number of implantation sites and number of corpora lutea. All fetuses were weighed and examined for external findings and sex. All fetuses were examined for soft tissue abnormalities and skeletal abnormalities.

Blood samples were obtained from satellite female rats on gestation day 20 on 0, 2, 4 and 6 hours after application of the cream. The live embryos were pooled per liter from satellite female rats for analysis of test article concentration after the last blood collection point. ASM 981 levels in blood samples and fetal tissue samples were determined by \_\_\_\_\_

The limit of quantification was  $\mu$ g/ml for blood samples and  $\mu$ g/g for embryonic tissue.

### Results:

- Mortality:** No treatment related effects on mortality were noted in this study.
- Clinical signs:** Some dermal irritation was noted in all treated groups (including vehicle) at the treatment site. No other treatment related effects on clinical signs were noted in this study.
- Body weight:** No treatment related effects on body weight were noted in this study.
- Food consumption:** No treatment related effects on food consumption were noted in this study.
- Gross Pathology:** No treatment related macroscopic findings were noted in this study.
- Toxicokinetics:** A summary of the toxicokinetic parameters (mean) is provided in the following table.

Dose (mg/kg/day)	C <sub>max</sub> (ng/ml)	T <sub>max</sub> (hr)	AUC <sub>0-6 hr</sub> (ng-hr/ml)	AUC <sub>0-24 hr</sub> (ng-hr/ml)	Embryo Conc. (ng/g)	Embryo/Plasma <sub>6 hr</sub> ratio
2 (0.2%)	0.5	6	1.1	5.6	1.9	3.8
6 (0.6%)	0.8	6	3.6	14.4	2.6	3.2
10 (1%)	1.4	6	5.9	24.8	7.2	5.1

An approximate dose dependent increase in ASM 981 systemic exposure was noted over the dose range tested in this study.

The embryo concentrations of ASM 981 increased ~4X over the dose range. The concentration ratios for embryonic tissue/maternal plasma at 6 hr ranged from 3.2 – 5.1 over the dose range tested. This ratio indicated good placental transfer of ASM 981 to the fetal compartment and that ASM 981 tended to concentrate in the embryo relative to the blood.

### **Female Reproductive Performance:**

No treatment related effects on female pregnancy rate, the number of implantation sites, the number of early and late resorptions, live and dead fetuses, number of implantation sites and number of corpora lutea were noted in this study. The sex ratio of male to female fetuses was not affected in any treatment group.

**Fetal Parameters:** No treatment related effect on fetal body weight was noted in this study. No treatment related external findings were noted in this study. No

treatment related soft tissue or skeletal malformations were noted in this study.

**Summary of individual study findings:**

No treatment related effects on maternal toxicity or female reproductive performance were noted in this study. The topical NOAEL for maternal toxicity and female reproductive performance is 10 mg/kg/day (1% ASM 981 cream; AUC<sub>0-6 hr</sub> = 5.9 ng-hr/ml; AUC<sub>0-24 hr</sub> = 24.8 ng-hr/ml). No signal of teratogenicity was noted in this study. The topical NOAEL for teratogenicity was 10 mg/kg/day (1% ASM 981 cream; AUC<sub>0-6 hr</sub> = 5.9 ng-hr/ml; AUC<sub>0-24 hr</sub> = 24.8 ng-hr/ml).

It is interesting to note that the level of systemic exposure after topical administration of the ASM 981 cream is greater in rabbits compared to rats. However, overall the level of systemic exposure in this dermal teratogenicity study is significantly less (12.5X lower) than noted in the oral teratogenicity study conducted in rabbits. Therefore, it is not surprising that only negative effects noted in this study. It is important to note that it would have been preferable if the daily treatment duration was for 24 hours instead of for 6 hours.

**Reproductive toxicology summary:**

The reproductive toxicity of ASM 981 was evaluated in fertility (rats), embryofetal developmental (rats and rabbits) and peri- and post-natal developmental (rats) oral studies. In addition, dermal teratogenicity (embryofetal developmental) studies were conducted in rats and rabbits.

Dermal administration of ASM 981 cream to rats and rabbits during the time of organogenesis was well tolerated and did not show any indication of embryotoxic or teratogenic potential. The NOAEL for embryotoxic and teratogenic effects in both studies was the highest dose tested (10 mg/kg/day; 1% ASM 981 cream; no AUC values in rat; AUC<sub>0-24 hr</sub> = 24.8 ng-hr/ml in rabbits). One possible reason for no effects being demonstrated in the dermal studies was due to the low levels of systemic exposure noted in both studies. Very low levels of systemic exposure (no AUC values could be calculated) were noted in the dermal teratogenicity study conducted in Wistar rats. This explains the negative effects noted in this study. The level of systemic exposure after topical administration of the ASM 981 cream was greater in rabbits compared to rats. However, overall the level of systemic exposure in the dermal rabbit teratogenicity study was significantly less (12.5X lower) than noted in the oral teratogenicity study conducted in rabbits. It is important to note that it would have been preferable if the daily treatment duration was for 24 hours instead of for 6 hours in both of the dermal teratogenicity studies.

Higher systemic exposure levels were reached in the oral reproductive toxicology studies. Fertility and general reproductive performance was assessed in the first part of the combined fertility and embryo-fetal development study in rats. No effect on mating or fertility was noted in males up to the dose of 45 mg/kg/day. However, females at this dose showed prolonged estrus cycle or were acyclic. The NOAEL for female fertility and general reproductive performance

was 10 mg/kg/day ( $AUC_{0-6hr} = 194$  ng·hr/ml; sponsor extrapolated  $AUC_{0-24hr} = 465$  ng·hr/ml). Compound related embryotoxicity as expressed by increased post implantation loss, reduced litter size, decreased fetal weights and increased rate of fetal retardation was noted at 45 mg/kg/day ( $AUC_{0-6hr} = 620$  ng·hr/ml; sponsor extrapolated  $AUC_{0-24hr} = 1448$  ng·hr/ml). The disturbances of the estrus cycle and possibly also the post implantation loss could potentially be attributed to lower levels of sex hormones demonstrated in a special toxicology study. The low fetal weights and retardation might be due to direct toxic effects of ASM 981. Oral administration of ASM 981 did not demonstrate any malformations up to a dose of 45 mg/kg/day in rats. However, the increased embryofetal lethality noted in the 45 mg/kg/day dose group could potentially serve as a possible teratogenic signal. ASM 981 crossed the blood-placental barrier in rats achieving mean embryonic tissue concentrations of 41 ng/g at the malformations NOAEL dose of 45 mg/kg/day (Maternal  $AUC_{0-6hr} = 620$  ng·hr/ml; sponsor extrapolated  $AUC_{0-24hr} = 1448$  ng·hr/ml).

No toxicologically relevant treatment related effects were noted in the oral embryo-fetal development study in rabbits. The NOAEL for maternal toxicity is 20 mg/kg/day ( $AUC_{0-6hr} = 74$  ng·hr/ml;  $AUC_{0-24hr} = 147$  ng·hr/ml), which was the highest dose tested in this study. No effects on the development of the embryos was observed at the highest dose level tested (20 mg/kg/day) in rabbits. ASM 981 crossed the blood-placental barrier in rabbits achieving mean embryonic tissue concentrations of 10 ng/g at the teratogenic NOAEL dose of 20 mg/kg/day (Maternal  $AUC_{0-6hr} = 74$  ng·hr/ml;  $AUC_{0-24hr} = 147$  ng·hr/ml).

Similar prenatal findings as were observed in the oral fertility and embryofetal developmental study in rats were noted in the oral rat pre-post natal development study. At the high dose of 40 mg/kg/day only 2 of 22 females delivered live pups. Postnatal survival, development of the F1 generation, their subsequent maturation and fertility were not affected by treatment up to the highest dose evaluated in this study (10 mg/kg/day). Therefore, the NOAEL for postnatal development was 10 mg/kg/day, which was the highest dose evaluated in this study. No AUC data was obtained in this study. However, the AUC data from the oral rat fertility and embryofetal developmental study could be used for this study since the 10 mg/kg/day dose level was tested in that study. Therefore, the AUC for the NOAEL for postnatal development can be set to equal the sponsor extrapolated value ( $AUC_{0-24hr} = 465$  ng·hr/ml) for labeling purposes.

### **Reproductive toxicology conclusions:**

Reproductive toxicology studies conducted in rats and rabbits, by dermal or oral administration, gave no signal for teratogenic potential for ASM 981. ASM 981 crossed the blood-placental barrier in both rats and rabbits. No adverse effects were noted on male fertility in rats. The fertility of female rats was impaired at 45 mg/kg/day. Suppressed estrogen levels might be the cause for disturbances of estrus cycle and possibly also for the embryoletality (implantation loss) in female rats at oral doses of >10 mg/kg/day. No reproductive toxicology effects were noted after dermal administration in rats and rabbits. However, the exposure duration in the dermal teratogenicity studies conducted in rats and rabbits was for 6 hr/day instead of the preferred 24 hr/day. Perhaps if the dermal teratogenicity studies had been conducted with a 24 hr/day exposure duration, higher systemic levels may have been achieved which may or may not have yielded different results in these studies.

**Labeling Recommendation:**

It is recommended that Elidel (pimecrolimus; ASM 981) cream be labeled as a Pregnancy C category drug. Embryotoxic effects were demonstrated in oral embryofetal developmental studies conducted in rats. Even though embryofetal lethality was achieved at doses that provided significant systemic exposure in rats, this could serve as a potential teratogenic signal for ASM 981. No embryotoxic or teratogenic effects were noted in the dermal teratogenicity studies conducted with the 1% ASM 981 cream in rats and rabbits. However, these studies were conducted with 6 hr/day exposure to the ASM 981 cream. It would have been preferable to have conducted these studies with a 24 hr/day exposure to the ASM 981 cream to maximize the systemic exposure in these studies. It is not anticipated that dermal administration of the 1% ASM 981 cream would be able to achieve systemic exposure levels in humans that would reach a level of concern for possible embryotoxic effects. Therefore, that is why it is recommended that Elidel cream be labeled as Pregnancy Category C

However, since ASM 981 clearly elicits embryofetal toxicity in rats after oral administration (which may potentially be a positive signal for teratogenicity), a pregnancy category C is more appropriate for the 1% ASM 981 cream.

**SPECIAL TOXICOLOGY (WITH FMF):****Special Toxicology Study #1:**

*Primary eye irritation study in rabbits*

Study title: Primary eye irritation study in rabbits

Key study findings: ASM 981 cream (1%) was minimally irritating in the rabbit eye.

Study no.: T-73/203-163  
Study No.: 649438  
Volume #, and page #: 48, 5-1  
Conducting laboratory: \_\_\_\_\_  
Date of study initiation: 2/17/97  
GLP compliance: Yes  
QA- Report: Yes (X) No ()  
Drug, and lot#: 1.0% ASM 981 cream – batch# Z061 0896  
Formulation/vehicle: Marketed cream formulation

Methods:

**APPEARS THIS WAY  
ON ORIGINAL**

ASM 981 cream (1%) (0.1 ml) was instilled into the conjunctival sac of the left eye of the animals and retained for 30 seconds. The right eye was not treated and served as a control to the treated eye.

Dosing:

- *species/strain:* New Zealand White rabbits
- *#/sex/group or time point:* 1 male and 2 females
- *age:* 17 weeks
- *weight:* male: 3.3 kg; females: 3.7-3.8 kg
- *dosage groups in administered units:* 0.1 ml of 1% ASM 981 cream
- *route, form, volume, and infusion rate:* route = intraocular

Observations and times:

- *Clinical Observations:* Daily
- *Body Weights:* Before treatment and before termination
- *Eye mucosa observations:* Injuries to the cornea, conjunctiva, sclera and iris were observed macroscopically in each animal 1, 24, 48 and 72 hours after instillation. The presence (or absence) of opacity, vascularization, reddening, edema, discharge, staining and test article remnants were recorded for each animal. Changes were evaluated according to the Draize standard.

Results:

- **Clinical Signs** No treatment related effects on clinical signs were noted in this study.
- **Body Weights** No treatment related effects on body weight were noted in this study.
- **Eye mucosa observations**

Slight to moderate reddening of the conjunctivae was noted in all animals and slight swelling of the conjunctivae was noted in 2/3 animals. All findings were reversible after 72 hours. The primary irritation score was 0.33

Summary of individual study findings:

ASM 981 cream (1%) was classified as minimally irritating in the rabbit eye.

**APPEARS THIS WAY  
ON ORIGINAL**

**Special Toxicology Study #2:***Determination of phototoxicity in guinea pigs*

**Study title:** Determination of phototoxicity in guinea pigs

**Key study findings:** The 1% ASM 981 cream and vehicle cream were classified as skin irritants in the guinea pig. No increase in the irritant reaction was noted after UV-A exposure.

**Study no.:** T-82/203-170  
**Study No.:** 652026  
**Volume #, and page #:** 49, 5-56  
**Conducting laboratory:** \_\_\_\_\_  
**Date of study initiation:** 3/26/97  
**GLP compliance:** Yes  
**QA- Report:** Yes (X) No ()  
**Drug, and lot#:** 1.0% ASM 981 cream – batch# Z061 0896  
**Formulation/vehicle:** Vehicle cream – batch# Z068 0896

**Methods:**

The animal's fur was shaved from both flanks of each animal with electric clippers the day before exposure. One test site of 2 cm<sup>2</sup> was marked on both flanks with a circular stamp. Test article was applied to the left flank of animals. The test article was applied with a spatula to saturate the test site. Thirty minutes after application of the test article, the left flank of the animals was exposed to non-erythemogenic UVA irradiation (20 J/cm<sup>2</sup>; \_\_\_\_\_ Lamps; Spectrum: 320 – 400 nm). After irradiation, test article was applied to the right flank of each animal. The right flank was not exposed to UVA irradiation.

**Dosing:**

- *species/strain:* Male Harley Guinea pigs
- *#/sex/group or time point:* 5 males/vehicle cream; 10 males/1% ASM 981 cream
- *age:* 5 – 7 weeks
- *weight:* 380 – 431
- *dosage groups in administered units:* refer to methods section above
- *route, form, volume, and infusion rate:* route = topical

**Observations and times:**

- *Mortality:* Daily
- *Clinical Signs:* Daily
- *Body Weights:* Daily
- *Dermal Signs:* Erythema and edema were assessed 24, 48 and 72 hours after exposure.

**Results:**

- **Mortality** No treatment related effects on mortality were noted in this study.
- **Clinical signs** No treatment related effects on clinical signs were noted in this study.
- **Body weights** No treatment related effects on body weight were noted in this study.
- **Dermal Signs** Slight to moderate skin reactions were noted on both the irradiated and non-irradiated sites. No potentiation of the skin reactions was noted at the irradiated sites.

Summary of individual study findings:

The 1% ASM 981 cream and vehicle cream were classified as skin irritants in the guinea pig. No increase in the irritant reaction was noted after UVA exposure. It is important to note that the design of this photoirritation study would typically not be acceptable since only UVA exposure was used in this study. It is preferable to conduct photoirritation studies with exposure to Visible/UVA/UVB exposure to mimic solar simulated conditions of exposure. However, it was determined that this study would not need to be repeated since clinical phototoxicity studies did not show an effect for Elidel cream.

Special Toxicology Conclusions:

The 1% ASM 981 cream was a minimal irritant in the rabbit eye. The photoirritation study conducted in guinea pigs under UVA exposure conditions did not demonstrate photoirritation. This result is not surprising since the drug product does not absorb in the VIS/UVA range. It would have been preferable if this study utilized UVB exposure, since ASM 981 showed absorption in the UVB range.

**IMPURITIES STUDIES:**

Background/Introduction:

The sponsor included information on the final levels of impurities that will be in the marketed 1% ASM 981 cream in the NDA submission. The following table is reproduced from the submitted information.

**APPEARS THIS WAY  
ON ORIGINAL**

According to the ICH guidance document titled "Q3B Impurities in New Drug Products", it is recommended to qualify any impurity found at a level  $\geq 0.1\%$  when a maximum daily dose of the drug product is  $> 2$  gm. The sponsor defines byproducts as those substances that are structurally related to the drug substance and formed during synthesis including           . The sponsor defines degradation products as those substances that are or may be produced due to decomposition of the compound in the drug product. The sponsor states that the weight percent of the impurities presented in the tables included in the NDA submission always refer to the drug substance also in the drug product as indicated in the ICH impurity guidelines. Therefore, the sponsor states that the concentration of the by- and degradation products in the ASM 981 cream is 100 times lower because the maximum achievable concentration of ASM 981 in the cream is 1%.

The sponsor has identified            potential ASM 981 by-products. They are listed below:

[ ]

The structures for each of the            potential ASM 981 by-products is provided below (scanned from the NDA submission).

WITHHOLD 1 PAGE (S)

The sponsor has identified ~~one~~ potential ASM 981 degradation products. They are listed below:

[ ]

The structures for each of the ~~one~~ potential ASM 981 degradation products is provided below (scanned from the NDA submission).

┌

└

**APPEARS THIS WAY  
ON ORIGINAL**

During the pre-NDA meeting (conducted on May 8, 2000), the sponsor was requested to submit the following information with the ASM 981 cream NDA submission concerning impurities of ASM 981.

- 1) List the level of each impurity (by-product or degradation product) that is expected for the final marketed formulation of the ASM 981 cream.
- 2) Submit all nonclinical toxicology studies that have been conducted to qualify the level of an impurity in the ASM 981 cream to the NDA.
- 3) Provide a clear rationale for the choice of nonclinical toxicology studies conducted for each impurity.
- 4) Provide a clear rationale for why appropriate nonclinical toxicology studies were not conducted for certain impurities in the ASM 981 cream.
- 5) Submit a table that lists the levels of impurities in the ASM 981 cream used in nonclinical long term toxicology studies. The levels of impurities should include the level of by-products and degradation products, if possible.

The sponsor included information to address the first request, which has been provided in tabular format previously. Information to address the fifth request was submitted to the NDA and is provided below (scanned from NDA submission). The next two tables provide data on the highest levels of impurities noted in repeat dose toxicity studies previously conducted for ASM 981.

**Table 1.1.-2. Batches and by-product levels used in repeated dose toxicity studies**

Drug substance batch	Dose mg/kg	By-products weight %	Studies	Doc.No.
_____	_____	_____	_____	_____
_____	_____	_____	_____	_____
_____	_____	_____	_____	_____
95907	1.5,25 2,8,30	_____	26-week, oral, Rat 26-week, oral, Mini pig	203-165 203-135

- 1) Cream Formulation B, see Table 4.-1
- 2) Oleogel, see Table 4.-2

It is important to note that the first three studies listed in this table were conducted with a different topical ASM 981 formulation than the to be marketed formulation. These studies did not undergo formal review for this NDA. Therefore, they will not be used for purposes of qualification of the impurities. Based on the data presented in this table the level of the \_\_\_\_\_ impurity was \_\_\_\_\_ in the 26 week oral rat study. The level of the \_\_\_\_\_ impurity in the 26 week oral rat study is about \_\_\_\_\_ that proposed for the to be marketed ASM 981 cream \_\_\_\_\_. The level of the \_\_\_\_\_ impurity was \_\_\_\_\_ in the 26 week oral rat study. The level of the \_\_\_\_\_ impurity in the 26 week oral rat study is about \_\_\_\_\_ that proposed for the to be marketed ASM 981 cream \_\_\_\_\_. The level of the \_\_\_\_\_ impurity in the 26 week oral rat study was \_\_\_\_\_. This level is at most \_\_\_\_\_ that proposed for the to be marketed ASM 981 cream \_\_\_\_\_. The level of the \_\_\_\_\_ impurity in the 26 week oral rat study was \_\_\_\_\_. This level is at most \_\_\_\_\_ that proposed for the to be marketed ASM 981 cream \_\_\_\_\_. The levels of impurities noted in the 26 week oral rat study may be adequate to qualify these impurities considering that a much greater exposure is anticipated via the oral route compared to the topical route.

**APPEARS THIS WAY  
ON ORIGINAL**

**Table 1.1.-3 Drug substance batches in the final market formulation with highest by-product levels used in relevant toxicity studies**

Drug subst. Batch	Dose mg/kg	By-products weight %	Studies	Doc.No.
<b>Release Limits</b>				
96914	10		26-week, dermal, Rat	203-216
(Z0040197	20		26-week, dermal, Mini pig	203-190
Z0841096 <sup>3)</sup> )			13-week, dermal, Rat	203-205
	10		13-week, dermal, juvenile Mini pig	203-191
	20		Embryotoxicity, dermal, Rat	203-210
	10		Embryotoxicity, dermal, Rabbit	203-203
96916	10		Photocarcinogenicity, Mouse	BS-119
(Z0250387 <sup>3)</sup> )	10		Carcinogenicity, dermal, Rat	682705

3) Final MF cream, see Table 4.1 in brackets: drug product batches

The only additional data that may help to qualify impurities in Table 1.1.-3 are the levels of \_\_\_\_\_ reported for the 26 week dermal rat study. The level of the \_\_\_\_\_ impurity \_\_\_\_\_ is about \_\_\_\_\_ the level proposed for the final to be marketed ASM 981 cream. \_\_\_\_\_ The level of the \_\_\_\_\_ impurity \_\_\_\_\_ is about \_\_\_\_\_ the level proposed for the final to be marketed ASM 981 cream. \_\_\_\_\_ These levels may be adequate to qualify these impurities.

The following two tables provide data on the level of impurities in separate toxicity studies conducted specifically for qualification of impurity levels. The referenced toxicity studies will be summarized later.

**Table 1.1.-5 Batches of SDZ ASM 981 spiked with by-products**

By-product	Weight %	Dose mg/kg	Type of Study	Species	Doc No.
		5, 10	2-week po	Rat	203-161
		5, 10	2-week po	Rat	203-161
			Ames	Salmonella	203-157
			Ames	Salmonella	BS-31
			V79 CA	Chin. Hamster	203-199
		5, 10	2-week po	Rat	203-214
		10	2-week po	Rat	203-214

CA = Chromosome aberration

**Table 1.2.-1 Potential degradation-products (weight % of drug substance) of SDZ ASM 981 used in toxicity studies**

Drug product batch	Drug subst. Batch	Type of Study	Species	Doc. No.
	99/1	Ames	Salmonella	BS-167
	99/1	V79 CA	Chin. Hamster	BS-148
	96/1	Ames	Salmonella	203-183
	96/1	V79 CA	Chin. Hamster	203-212
	96/1	2-week, po	Rat	203-202
Z0660866*		2-week, derm.	Rat	203-208

\*Stressed cream: impurity profile after storage conditions of 12 months at 40°C  
 CA = Chromosome aberration

The sponsor conducted several studies (pharmacology, mutagenicity and 2 week repeat dose toxicity studies) to supply additional data for the qualification of the impurities in ASM 981. These studies are summarized below.

**Impurities Studies Summary:**

Several of the potential by-products (formed during the \_\_\_\_\_ process) were evaluated in nonclinical toxicology studies. The by-products are structurally similar to the drug substance. The by-products of major concern are those that will have a concentration \_\_\_\_\_ in the final marketed 1% ASM 981 cream. These by-products include \_\_\_\_\_

\_\_\_\_\_ were qualified by conducting 2 week oral rat studies, AMES test and chromosomal aberration assays with either \_\_\_\_\_ ASM 981 or \_\_\_\_\_ ASM 981. For both impurities, the toxicity profile of the spiked samples were equivalent to ASM 981 in the 2 week oral rat studies and both the AMES and chromosomal aberration assays were negative for both impurities. The sponsor states in the submission that the by-products \_\_\_\_\_ were detected only in later batches of ASM 981. Therefore, it was determined by the sponsor to conduct the previously described studies to qualify these impurity levels. The level of the \_\_\_\_\_ impurity was \_\_\_\_\_ in the ASM 981 cream batch used in the 26 week dermal rat and minipig studies and \_\_\_\_\_ in the ASM 981 cream batch used in the mouse photocarcinogenicity study and the dermal

rat carcinogenicity study. The level of the \_\_\_\_\_ impurity was \_\_\_\_\_ in the ASM 981 cream batch used in the 26 week dermal rat and minipig studies and \_\_\_\_\_ in the mouse photocarcinogenicity study and the dermal rat carcinogenicity study. Since \_\_\_\_\_ is an \_\_\_\_\_ of ASM 981 similar to \_\_\_\_\_, the impurity studies conducted qualify the \_\_\_\_\_ impurity as well. The level of the \_\_\_\_\_ impurities used in the spiked oral and genotoxicity studies along with the level noted in the long term dermal toxicity/carcinogenicity studies qualify the level proposed for the final marketed 1% ASM 981 cream.

The \_\_\_\_\_  
impurities were qualified based on amounts identified in nonclinical studies conducted with ASM 981. The sponsor is seeking an impurity level of \_\_\_\_\_ for the \_\_\_\_\_ impurity. A level of \_\_\_\_\_ was identified in the ASM 981 batch used for both 26 week oral rat and minipig toxicity studies. A level of \_\_\_\_\_ was identified in the ASM 981 batch used in the oral mouse micronucleus test. Since all of these studies were oral and the exposure to the \_\_\_\_\_ impurity would be much higher via the oral route than via the dermal route, these studies are adequate to qualify the \_\_\_\_\_ impurity at the \_\_\_\_\_ level in relation to systemic toxicity. The level of \_\_\_\_\_ impurity was \_\_\_\_\_ in the ASM 981 cream batch used in the 26 week dermal rat and minipig studies and \_\_\_\_\_ in the mouse photocarcinogenicity study and the dermal rat carcinogenicity study. These levels are adequate to qualify the \_\_\_\_\_ impurity concerning potential dermal toxicity for the level proposed for the final marketed 1% ASM 981 cream.

The sponsor is seeking an impurity level of \_\_\_\_\_ for the \_\_\_\_\_ impurity. A level of \_\_\_\_\_ was identified in the ASM 981 batch used for both 26 week oral rat and minipig toxicity studies. A level of \_\_\_\_\_ was identified in the ASM 981 batch used in the oral mouse micronucleus test. The level of \_\_\_\_\_ impurity was \_\_\_\_\_ in the ASM 981 cream batch used in the 26 week dermal rat and minipig studies and \_\_\_\_\_ in the mouse photocarcinogenicity study and the dermal rat carcinogenicity study. The levels in the oral toxicity studies and dermal toxicity studies are adequate to qualify the \_\_\_\_\_ impurity concerning potential systemic and dermal toxicity for the level proposed for the final marketed 1% ASM 981 cream.

The sponsor is seeking an impurity level of \_\_\_\_\_ for the \_\_\_\_\_ impurity. A level of \_\_\_\_\_ was identified in the ASM 981 batch used for both 26 week oral rat and minipig toxicity studies. A level of \_\_\_\_\_ was identified in the ASM 981 batch used in the oral mouse micronucleus test. The level of \_\_\_\_\_ impurity was \_\_\_\_\_ in the ASM 981 cream batch used in the 26 week dermal rat and minipig studies and \_\_\_\_\_ in the mouse photocarcinogenicity study and the dermal rat carcinogenicity study. The levels in the oral toxicity studies and dermal toxicity studies are adequate to qualify the \_\_\_\_\_ impurity concerning potential systemic and dermal toxicity for the level proposed for the final marketed 1% ASM 981 cream.

The only impurity that did not undergo formal nonclinical toxicity testing is the \_\_\_\_\_ impurity. The sponsor is seeking an impurity level of \_\_\_\_\_ for the \_\_\_\_\_ impurity. Unfortunately, the sponsor did not include a graphic of the \_\_\_\_\_ impurity in the submission. However, a chemical name was included in the chemistry portion of the NDA submission. I was able to obtain this information from the chemistry reviewer, Ernie Pappas. The chemical name for the \_\_\_\_\_ impurity is:

The \_\_\_\_\_ impurity was not identified in any of the ASM 981 oral batches or dermal cream batches used in the nonclinical toxicity studies. However, it was identified at \_\_\_\_\_, in clinical batches 990001 and 990002 and at \_\_\_\_\_ in clinical batches 990003, 990004, 990005 and 990006 used in clinical studies. Since the \_\_\_\_\_ impurity was noted in clinical batches used in clinical studies, this provides adequate studies to qualify the \_\_\_\_\_ impurity concerning potential dermal toxicity for the level proposed for the final marketed 1% ASM 981 cream.

Degradation products are produced due to decomposition of the compound in the drug product. Several degradation products were identified during stability studies. The sponsor states that the 1% ASM 981 cream formulation was fairly stable in \_\_\_\_\_ tubes at 25°C and 60% relative humidity for up to 2 years. Under these conditions, only one degradation product \_\_\_\_\_ reached a concentration of about \_\_\_\_\_ whereas \_\_\_\_\_ reached about \_\_\_\_\_. Under accelerated conditions (30°C and 60% relative humidity or 40°C and 75% relative humidity) higher amounts of these degradation products were observed whereas \_\_\_\_\_ appeared in small amounts. The release limits that the sponsor states in the submission for degradation products is \_\_\_\_\_.

The sponsor identifies the impurities \_\_\_\_\_ as potential by products and degradation products. The levels for these two impurities are listed in the level of by-products in the drug substance provided in the background section to this portion of the review. Adequate nonclinical toxicity studies have been conducted to support the \_\_\_\_\_ impurity. However, the \_\_\_\_\_ impurity was not studied in previous described nonclinical toxicity studies. The release limit levels of the degradation products identified in the 1% ASM 981 cream are in addition to the levels of by-products in the drug substance. The sponsor states that since the storage conditions of ASM 981 cream are more stringent during development than after launch only low levels of degradation products were examined in the standard toxicity program or clinical studies. Therefore, the sponsor conducted separate studies to cover the release limits for the degradation products.

The mutagenicity tests (AMES and chromosomal aberration assay) conducted with ASM 981 spiked with the degradation products \_\_\_\_\_ were negative. No additional toxicity was noted in a 2 week oral rat study conducted with ASM 981 spiked with \_\_\_\_\_. The sponsor states that because the systemic absorption of the degradation products is considered to be equally low as for ASM 981, the evaluation of the degradation products focussed on the local effect on the skin. Therefore, the irritation potential of stressed (40°C for 12 months) 1% ASM 981 cream was tested in a 2 week dermal toxicity study in rats. No signs of skin irritation were noted in this study. The level of the degradation products in the stressed 1% ASM 981 cream used in this study were \_\_\_\_\_. The studies conducted by the \_\_\_\_\_.

sponsor for the degradation products of 1% ASM 981 cream are adequate to qualify the levels of the degradation products at the designated release limits.

**Impurities Studies Conclusions:**

The sponsor conducted adequate nonclinical toxicity studies or clinical studies to qualify the level of by-products and degradation products proposed for the to be marketed formulation of 1% ASM 981 cream.

**APPEARS THIS WAY  
ON ORIGINAL**

**This is a representation of an electronic record that was signed electronically and  
this page is the manifestation of the electronic signature.**

/s/  
-----

Barbara Hill  
9/25/01 07:19:23 AM  
PHARMACOLOGIST

Abby Jacobs  
9/25/01 07:26:13 AM  
PHARMACOLOGIST

Jonathan Wilkin  
9/25/01 09:32:56 AM  
MEDICAL OFFICER

**APPEARS THIS WAY  
ON ORIGINAL**

## REVIEW AND EVALUATION OF PHARMACOLOGY/TOXICOLOGY DATA:

**KEY WORDS:** Immunosuppressant, Atopic Dermatitis, ASM 981, Mouse and Rat Dermal Carcinogenicity Studies, Addendum Review

**Reviewer Name:** Barbara Hill

**Division Name:** Dermatologic and Dental Drug Products

**HFD#:** HFD-540

**Review Completion Date:** 9-12-01

**NDA number:** 21-302

**Note:** This review is an addendum to the original review. This document contains the review of the mouse and rat dermal carcinogenicity studies only.

**Serial number/date/type of submission:** 000 / 12-19-00 / Original NDA Submission

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

**Sponsor:** Novartis Pharmaceuticals Corporation  
59 Route 10  
East Hanover, New Jersey 07936  
(973) 781-7548

**Manufacturer for drug substance:** Novartis Pharma AG  
Lichtstrasse 35  
CH-4056 Basle, Switzerland

### Drug:

**Code Name:** ASM 981 Cream, 1%

**Generic Name:** Pimecrolimus

**Trade Name:** Elidel™

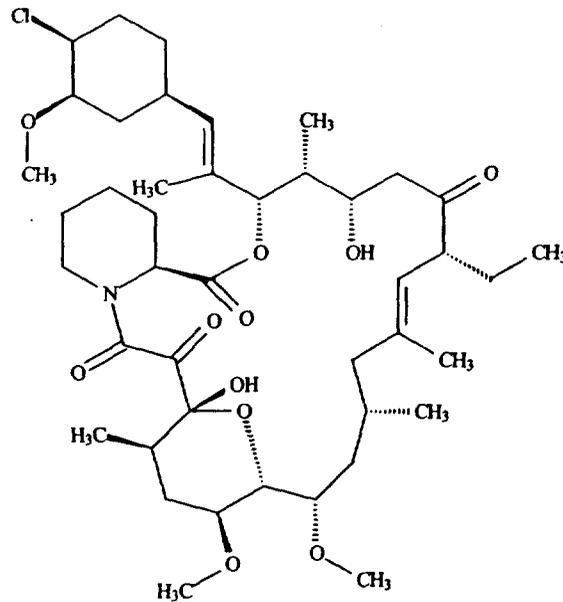
**Chemical Name:** (1R, 9S, 12S, 13R, 14S, 17R, 18E, 21S, 23S, 24R, 25S, 27R)-12-[(1E)-2-[(1R, 3R, 4S)-4-chloro-3-methoxycyclohexyl]-1-methylvinyl]-17-ethyl-1, 14-dihydroxy-23, 25-dimethoxy-13, 19, 21, 27-tetramethyl-11, 28-dioxo-4-aza-tricyclo[22.3.1.0<sup>4,9</sup>]octacos-18-ene-2, 3, 10, 16-tetraone

**CAS Registry Number:** 137071-32-0

**Molecular Formula/ Molecular Weight:** 810.47 / C<sub>43</sub>H<sub>68</sub>ClNO<sub>11</sub>

**UV Absorption:**  $\lambda_{max}$  (~ 200  $\mu$ g/ml in methanol or ethanol): — nm ( $\epsilon =$  — )

**Note:** Only an absorption spectra for the active, ASM 981, has been provided for ASM 981 cream. The sponsor has provided no information on UVA/B or visible absorption for the inactive ingredients in the drug product.

**Structure:****Relevant INDs/NDAs/DMFs:**

- 1) IND — (1% ASM 981 cream, Atopic Dermatitis; HFD-540)
- 2) IND [
- 3) IND [
- 4) IND ]

**Drug Class:** Anti-inflammatory, immunosuppressant

**Indication:** Atopic Dermatitis

**Clinical formulation:**

The composition of the 1% ASM 981 cream (the final clinical formulation) is provided in the following table:

**APPEARS THIS WAY  
ON ORIGINAL**

Ingredient	Amount per gram of Drug Product (mg)	Function
ASM 981	10.0	Drug Substance
Sodium Hydroxide	{	
Citric acid		
Benzyl alcohol		
Sodium cetostearyl sulphate		
Cetyl alcohol		
Stearyl alcohol		
Propylene glycol		
Oleyl alcohol		
Triglycerides		
Water		

**Dose:**

The sponsor provided the following information for a request of anticipated maximum dose for 1% ASM 981 cream.

- Up to 60-75% total body surface area will be treated in both adult and pediatric patients
- The maximum amount of ASM 981 cream, 1% to be applied per application is approximately 15-20 grams.
- Frequency of application is BID (twice daily)

Based on this information the maximum daily dose of 1% ASM 981 cream would deliver 0.4 gm of active ingredient ( $20 \text{ gms} \times .01 \times 2/\text{day} = 0.4 \text{ gm/day}$ ). For a 50 kg person, this dose would be 8 mg/kg/day.

Note: It is estimated that up to 80% of the body could be treated in a severe case of atopic dermatitis. Approximately 30 g of 1% ASM 981 cream could be applied per treatment to cover 80% of the body. Therefore, the maximum daily dose of the 1% ASM 981 cream would be 12 mg/kg/day for a 50 kg person ( $30,000 \text{ mg} \times .01 \times 2/\text{day} + 50 \text{ kg} = 12 \text{ mg/kg/day}$ ). This estimate is 1.5 fold greater than the estimate based on the data provided by the sponsor. It is recommended that this estimate be used for calculation of fold human exposure levels based on nonclinical toxicity studies.

**Route of administration:** Topical dermal

**Disclaimer:** Note some material may be taken directly from sponsor's submission.

**Introduction and drug history:**

The oral mouse and rat carcinogenicity studies final study reports were submitted under IND ~~\_\_\_\_\_~~.

The dermal mouse and rat carcinogenicity study final study reports were submitted for the first time with the NDA submission. The review for both the dermal mouse and rat carcinogenicity studies will be performed in this addendum review.

Human pharmacokinetic studies have been performed with the 1% ASM 981 cream in adult and pediatric patients under maximum use conditions. Adult and pediatric patients treated dermally with daily bid 1% ASM 981 cream showed blood levels mostly below 1 ng/ml or near the limit of quantification. The systemic exposure to ASM 981 in clinical topical studies with the 1% ASM 981 cream is provided in the following table. This human pharmacokinetic data was provided in the NDA 21-302 submission.

Study (patient population)	Range of treated body surface area (%)	Maximum Drug concentration (ng/ml)	AUC <sub>(0-12 hr)</sub> range (ng·hr/ml)
0304 (3-24 months)	10 – 92	2.26	not calculated
0301 (4-11 months)	25 – 58	2.6	not calculated
W204 (adults)	15 – 59	1.4	0 – 11.4
W202 (1-4 years)	23 – 69	1.8	0 – 18.8
W206 (8-14 years)	21 – 50	2.0	0 – 16.4
W205 (adults)	14 – 62	0.8	not calculable <sup>a</sup>

a – 95% of the concentration values below limit of quantitation (— ng/ml)

The highest AUC<sub>(0-12 hr)</sub> for a pediatric patient treated on 43.5% body surface area (BSA) was 18.8 ng·hr/ml. The AUC<sub>(0-24 hr)</sub> for this pediatric patient would be 2 x AUC<sub>(0-12 hr)</sub> since the 1% ASM 981 cream is applied bid. Therefore, the highest AUC<sub>(0-24 hr)</sub> for a pediatric patient was 38 ng·hr/ml (2 x 18.8 ng·hr/ml). The highest AUC<sub>(0-12 hr)</sub> for an adult patient treated on 59% BSA was 11.4 ng·hr/ml. The AUC<sub>(0-24 hr)</sub> for this adult patient would be 2 x AUC<sub>(0-12 hr)</sub> since the 1% ASM 981 cream is applied bid. Therefore, the highest AUC<sub>(0-24 hr)</sub> for an adult patient was 23 ng·hr/ml (2 x 11.4 ng·hr/ml). Since the highest AUC<sub>(0-24 hr)</sub> was observed in a pediatric patient (38 ng·hr/ml), this will be the value used to calculate the multiple of human exposure ranges for the oral mouse and oral rat carcinogenicity studies.

**Studies reviewed within this submission:**

(Note: The mouse and rat dermal carcinogenicity studies are reviewed in this addendum review. The rest of the nonclinical pharmacology/toxicology studies are reviewed in the original review.)

- 1) Carcinogenicity study by dermal administration in mice
- 2) 104-Week dermal carcinogenicity study in rats