

study. 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.

The sponsor identified potential by-products (formed during the \_\_\_\_\_ and degradation products (produced \_\_\_\_\_ in the final to be marketed Elidel™ cream. The by-products and the degradation products are structurally similar to the drug substance. 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 Elidel™ cream (1% ASM 981 cream).

#### **Safety issues relevant to clinical use:**

The repeat dose oral and dermal toxicity studies conducted in mice, rats and minipigs are adequate to determine the nonclinical toxicity profile for ASM 981. The duration of treatment is adequate to support clinical use (26 weeks in oral and dermal rat studies and 26 weeks in oral and dermal minipig studies). It would have been preferable to have conducted 9 month oral and dermal minipig studies to support the ASM 981 cream. However, it was decided early in the development that 26 week oral and dermal studies would be adequate for the ASM 981 cream. Also, additional long-term nonclinical toxicity data is available from oral carcinogenicity studies conducted in rats and mice. \_\_\_\_\_

Results for repeat dose (26 weeks) oral toxicity studies conducted in rats demonstrate that rodents are more sensitive to ASM 981 than non-rodents. Lymphoreticular effects (i.e., reduced lymphocyte counts and medullary atrophy in the thymus) were noted in rats that were a direct consequence of the immunosuppressive activity of ASM 981. Toxicological effects of ASM 981 in rats included functional and/or morphological changes noted in the kidney and pancreas at high doses of ASM 981. An increased incidence of lens cataracts was noted after chronic oral treatment with high doses of ASM 981. ASM 981 also showed effects on reproductive organs at high doses. Specifically noted were reduced prostate gland weight, epithelial atrophy of seminal vesicles, suppression of estrus cycle, vaginal and uterine atrophy.

No systemic toxicity was noted in the 26 week dermal toxicity study in rats. This was not surprising due to the very low level of systemic exposure to ASM 981 achieved in this study. However, the design of the study was adequate since use of the maximum feasible concentration of the ASM 981 cream and maximum amount to be applied topically was used in this study.

The multiple of human exposure (which will be used to estimate potential human risk) will be calculated from all nonclinical data provided in this section based on the highest measured  $AUC_{(0-24 \text{ hr})}$  value measured in humans that applied 1% ASM 981 cream. This  $AUC_{(0-$

<sup>24 hr</sup>) value was 38 ng-hr/ml and was measured in a single pediatric patient that applied 1% ASM 981 cream bid to 43.5% BSA. The NOAEL identified in the 26 week repeat dose oral toxicity study in rats was 1 mg/kg/day ( $AUC_{0-24 \text{ hr}} = 17.5$  and  $23.7$  ng-hr/ml for males and females, respectively). The multiple of human exposure based on the NOAEL in the 26 week repeat dose toxicity study in rats is 0.46 – 0.62X. Typically this low of a multiple of human exposure would be cause for concern. However, there are data in non-rodents (described below) that suggest that perhaps rodents are more sensitive to the toxicity associated with ASM 981 than are humans. In addition, it must be taken into consideration that the majority of the human pharmacokinetic measurements after repeat dose administration of the 1% ASM 981 cream were below the level of detection. Therefore, the actual potential risk of the toxicities identified in the rat study may be much less than the estimate calculated based on the highest measured  $AUC_{(0-24 \text{ hr})}$  value measured in humans that applied 1% ASM 981 cream. It is important to note that the sponsor did include safety monitoring in the clinical studies for kidney and pancreas based on the toxicities identified in the rat toxicity study.

Potential target organs of toxicity identified in the 26 week oral toxicity study in minipigs included the arteries, adrenals and lungs in minipigs. Arteries were considered the major target organ for toxicity in minipigs. Damage to the arteries (arteritis) in the adrenals was noted in all high dose animals and in various other organs in some of the high dose animals. It would appear that arteritis became more prominent in minipigs after treatment with ASM 981 for a longer duration of treatment. The NOAEL identified in this study was 2 mg/kg/day ( $AUC_{0-24 \text{ hr}} = 316$  and  $305$  ng-hr/ml for males and females, respectively) for minipigs after 26 weeks of oral administration of ASM 981. The multiple of human exposure based on the NOAEL in the 26 week repeat dose toxicity study in minipigs is ~8X.

No systemic or dermal toxicity was noted in the 26 week dermal toxicity study in minipigs. This was not surprising due to the very low level of systemic exposure to ASM 981 achieved in this study. However, the design of the study was adequate since use of the maximum feasible concentration of the ASM 981 cream and maximum amount to be applied topically were used in this study.

Repeat dose toxicity studies conducted in mice determined one of the major concerns associated with long term use of ASM 981, which would be the formation of malignant lymphoma due to overt systemic immunosuppression. Malignant lymphoma was noted as early as 8 weeks at high dermal doses of ASM 981 (100 mg/kg/day; dissolved in ethanol). A 13 week dermal toxicity study conducted for ASM 981 determined that the NOAEL for lymphoproliferative changes was identified in this study as 10 mg/kg/day ( $AUC_{0-24 \text{ hr}} = 643$  and  $675$  ng-hg/ml for males and females, respectively) for mice after 13 weeks of topical administration of ASM 981 dissolved in ethanol.

Malignant lymphoma was noted as a treatment related neoplastic lesion in the oral mouse carcinogenicity study. The NOAEL for lymphoma formation was identified as the 15 mg/kg/day dose group ( $AUC_{(0-24 \text{ hr})}$  for males =  $2260$  ng-hr/ml after week 70 of treatment;  $AUC_{(0-24 \text{ hr})}$  for females =  $5059$  ng-hr/ml after week 70 of treatment) in this study. The multiple of human exposure for the oral mouse carcinogenicity study ranged from 60 – 133X for lymphoma

formation based on the NOAEL  $AUC_{(0-24 \text{ hr})}$  levels. This provides an adequate safety margin for the potential formation of lymphoma.

Unfortunately the dermal mouse carcinogenicity study (conducted with ASM 981 dissolved in ethanol) did not use an adequate dose range. The sponsor selected a high dose group (4 mg/kg/day; average  $AUC_{(0-24 \text{ hr})} = 1080$  ng-hr/ml after 52 weeks of treatment) in the dermal mouse carcinogenicity study that would not cause lymphoma formation. Therefore, the results from this study were negative. However, enough data exist from other repeat dose dermal toxicity studies in mice to better define the dose range for the potential formation of lymphoma in mice. The NOAEL for lymphoproliferative changes was identified in a 13 week repeat dose study as 10 mg/kg/day ( $AUC_{0-24\text{hr}} = 643$  and 675 ng-hg/ml for males and females, respectively) for mice after 13 weeks of topical administration of ASM 981 dissolved in ethanol. The lowest dose that a low incidence of lymphoproliferative changes was identified in this study was 25 mg/kg/day ( $AUC_{0-24\text{hr}} = 1845$  and 1745 ng-hg/ml for males and females, respectively) for mice after 13 weeks of topical administration of ASM 981 dissolved in ethanol. Even though the dermal mouse carcinogenicity study was not conducted with an adequate dose range selection, the NOAEL dose in this study (4 mg/kg/day) did provide for a multiple of human exposure equal to 28X based on the NOAEL  $AUC_{(0-24 \text{ hr})}$  levels identified in this dermal mouse carcinogenicity study. This multiple of human exposure suggests an adequate safety margin for the potential concern of lymphoma formation in humans after use of 1% ASM 981 cream under maximum use conditions.

A positive signal for benign thymoma was noted in the oral rat carcinogenicity studies conducted with ASM 981. Combining the results of the two oral rat carcinogenicity studies, the NOAEL for benign thymoma formation is 1 mg/kg/day in male rats ( $AUC_{(0-24 \text{ hr})}$  for males = 42 ng-hr/ml after week 72 of treatment) and 5 mg/kg/day in female rats ( $AUC_{(0-24 \text{ hr})}$  for females = 805 ng-hr/ml after week 72 of treatment). The multiple of human exposure for the oral rat carcinogenicity study ranged from 1.1 – 21X based on the NOAEL  $AUC_{(0-24 \text{ hr})}$  levels. In my opinion, the multiple of human exposure based on the NOAEL for female rats (21X) does provide for an adequate safety margin for the potential concern of benign thymoma formation in humans after use of 1% ASM 981 cream under maximum use conditions. However, the multiple of human exposure based on the NOAEL for male rats (1.1X) does not provide for an adequate safety margin. However, it must be taken into consideration that the majority of the human pharmacokinetic measurements after repeat dose administration of the 1% ASM 981 cream were below the level of detection. Therefore, the actual potential risk of thymoma formation may be much less than the estimate calculated based on the highest measured  $AUC_{(0-24 \text{ hr})}$  value measured in humans that applied 1% ASM 981 cream.

The dermal rat carcinogenicity study that was conducted with the final to be marketed ASM 981 cream product did not show a positive signal for carcinogenicity. The NOAEL identified in this study is 10 mg/kg/day (average  $AUC_{(0-24 \text{ hr})} = 125$  ng-hr/ml after 104 weeks of treatment). The multiple of human exposure is 3.3X based on the NOAEL  $AUC_{(0-24 \text{ hr})}$  levels identified in this dermal rat carcinogenicity study. The highest dose feasible was tested in the dermal rat carcinogenicity study. Therefore, the study was determined to be adequate.



Based on the nonclinical data available for oral pimecrolimus and pimecrolimus cream, my recommendation for NDA 21-302 is that it be approvable from a pharmacology/toxicology perspective provided that the recommended changes in the label discussed in the next section are incorporated into the label.

**Labeling Review:**

The entire Elidel<sup>TM</sup> label is inserted below. The version of the label that is reviewed below was submitted to the NDA as a labeling supplement dated 6-21-01. Comments about the portions that relate to nonclinical pharmacology/toxicology will be inserted directly in the appropriate sections. Recommended sections to be deleted are marked by ~~strikeout~~. Recommended sections to be added are marked by highlight.

Note: Reviewer's comments in support of recommended labeling changes are provided in *italics*.

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Draft

Labeling

**RECOMMENDATIONS:**

**Internal comments:**

Based on the nonclinical data available for oral pimecrolimus and pimecrolimus cream, my recommendation for NDA 21-302 is that it be approvable from a pharmacology/toxicology

perspective provided that the recommended changes in the label discussed in the previous section are incorporated into the label.

**NDA issues:**

An outstanding information need for reanalysis of the histopathology of the low and mid dose animals for the thymus and thyroid in the rat dermal carcinogenicity study still needs to be addressed. Based on the data available for the rat dermal carcinogenicity study, the results appear to be negative. A reanalysis will be performed when the additional data requested is submitted to the NDA. If the data demonstrate that there is an additional concern for this study, then this will be recommended for inclusion in the label in an addendum review to the NDA.

Barbara Hill, Ph.D.  
Reviewing Pharmacologist

cc:

NDA: 21-302 (000)

HFD-340

HFD-540/DIV FILES

HFD-540/TOX/JACOBS

HFD-540/PHARM/HILL

HFD-540/MO/COOK

HFD-540/CHEM/PAPPAS

HFD-540/PM/WRIGHT

Concurrence Only:

HFD-540/DivDir/JWILKIN

HFD-540/PharmSup/AJACOBS

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**APPEARS THIS WAY  
ON ORIGINAL**

**STUDIES REVIEWED WITHIN THIS SUBMISSION:**

Note: FMF refers to the final marketed formulation for 1% ASM 981 cream

**Nonclinical Pharmacokinetic Studies:****Mouse ADME:**

- 1) Absorption and disposition of [<sup>3</sup>H]ASM981 in mouse following a single oral dose of 15 mg/kg and comparison with a single intravenous reference dose of 3 mg/kg (M-7/R98-491)

**Rat ADME:**

- 2) Absorption and disposition in rat following single 6.17 μmol/kg (5.0 mg/kg) oral, 123.4 μmol/kg (100 mg/kg) oral, 1.23 μmol/kg (1.0 mg/kg) intravenous and multiple 3.7 μmol/kg/day (3 mg/kg/day) topical doses (Formulation B) of [<sup>3</sup>H]SDZ ASM 981 (M-8/303-132)
- 3) Supplementary metabolism study in the rat with administration of a single oral dose of 100 mg/kg [<sup>3</sup>H]ASM981 (M-9/R98-444)
- 4) \_\_\_\_\_ in albino and pigmented rats after po and iv administration of [<sup>3</sup>H]ASM981 (M-10/303-200)
- 5) Embryo-fetal transfer in pregnant rats on day 13 and on day 17 of gestation after po administration of [<sup>3</sup>H]ASM981 (M-11/303-201)
- 6) Embryo-fetal transfer in pregnant rats on day 13 and day 17 of gestation after po administration of [<sup>3</sup>H]ASM981. Supplementary biotransformation data (M-12/R98-375-01)

**Rabbit ADME:**

- 7) Placental transfer in rabbits after peroral administration of 20 mg/kg of [<sup>3</sup>H]ASM981 (M-13/R98-494)

**Minipig ADME:**

- 8) Absorption and disposition in minipig following single 5.7 μmol/kg (4.65 mg/kg) or 48.7 μmol/kg (39.6 mg/kg) oral (solid dispersion) and 2.3 μmol/kg (1.85 mg/kg) intravenous doses of [<sup>3</sup>H] SDZ ASM 981 (M-14/303-133)
- 9) Absorption and disposition in minipigs after a single topical (dermal) application [<sup>3</sup>H]-labeled SDZ ASM 981 formulated as 1% FMF cream (M-15/303-195)

**In Vitro Blood/Plasma Partitioning and Protein Binding Studies:**

- 10) *In vitro* distribution and stability of [<sup>3</sup>H] SDZ ASM 981 in rat, mini-pig and human blood (M-16/303-039)
- 11) *In vitro* protein binding of [<sup>3</sup>H]ASM981 in rats, minipigs and humans (M-18/303-211)

- 12) ASM 981: *In vitro* blood distribution and protein binding of <sup>3</sup>H-labeled ASM981 in mouse and rabbit and stability in mouse and rabbit blood (M-17/R00-1510)

*In Vitro* Metabolism Studies:

- 13) Metabolism by rat and human liver microsomes and by human liver S12 fractions (M-19/R97-541)
- 14) Metabolism by rat and human liver microsomes and by human liver S12 fractions, Amendment No. 1 (M-20/R97-541-01)
- 15) CYP3A4/5 is the main cytochrome P450 isozyme involved in the microsomal biotransformation of ASM 981 (M-21/R98-1075)
- 16) Species comparison of hepatic metabolism *in vitro* (M-22/R98-192)
- 17) Evaluation of ASM981 as an inhibitor of human P450 enzymes (M-23/R97-532)
- 18) Tautomer interconversion of ASM981 and metabolites (M-24/R99-2683)
- 19) Metabolism of ASM981 in human skin *in vitro* (M-25/R00-1030)
- 20) Pharmacological activity of human metabolite pools (M-26/R99-2221)
- 21) Mechanistic transport studies across Caco-2 cell monolayers (M-27/R00-1674)

Repeat Dose Toxicology Studies:

Oral Mouse:

- 1) Tissue concentration following a 2 week oral (solid dispersion) and dermal treatment (ethanol solution) in mice (T-18/203-180)
- 2) A 13-week oral (per gavage) dose-range-finding study in mice (Drug form: lyophilisate suspension) (T-10/203-164)

Dermal Mouse FMF:

- 3) A 4-week dermal toxicokinetic study of SDZ ASM 981 cream administered to hairless mice (T-12/203-197)
- 4) Range-finding tolerance test of SDZ ASM 981 administered topically to hairless mice for 8 weeks (T-13/203-189)

Dermal Mouse non-FMF:

- 5) Preliminary toxicity study by dermal administration to CD-1 mice for 13 weeks followed by a 4 week reversibility period (Drug form: Ethanol solution) (T-19/203-136)
- 6) Study to investigate the dosage response of immunosuppression and lymphoproliferative disorders following dermal administration to CD-1 mice for 13 weeks (T-89/203-181)
- 7) Study to investigate the severity of immunosuppression and the rate of onset of lymphoproliferative disorders following dermal administration to CD-1 mice for 13 weeks (T-90/203-192)
- 8) Investigational dermal painting study in mice: 1, 2, 3-month treatment with a 13 week recovery period (T-91/203-207)
- 9) Oncogenicity study by dermal administration to CD-1 mice for 52 weeks (T-92/203-182)

**Oral Rat:**

- 10) 4-week oral reproductive hormone study in male rats (T-95/BS-728)
- 11) 4-week oral reproductive hormone study in female rats (T-96/BS-730)
- 12) A 13-week oral (per gavage) toxicity study in rats (Drug form: Lyophilisate suspension) (T-26/203-095)
- 13) A 26-week oral (per gavage) toxicity study in rats (Drug form: solid dispersion) (T-27/203-165)

**Dermal Rat FMF:**

- 14) A 13-week dermal toxicity study in rats (T-30/203-205)
- 15) A 26-week dermal toxicity study in rats (T-31/203-216)

**Oral Minipig:**

- 16) Two-week oral (gavage) toxicity study in the minipig (Drug form: lyophilisate suspension) (T-40/203-042)
- 17) A 2-week oral (gavage) toxicity study in minipigs (Drug form: solid dispersion) (T-43/203-126)
- 18) A 2-week oral dose range finding study in juvenile minipigs (Drug form: solid dispersion) (T-44/203-154)
- 19) 4-week oral (gavage) toxicity study in juvenile minipigs (Drug form: solid dispersion) (T-46/203-137)
- 20) 4-week oral (gavage) toxicity study in minipigs (Drug form: lyophilisate suspension) (T-47/203-047)
- 21) A 26-week oral (gavage) toxicity study in minipigs (Drug form: solid dispersion) (T-50/203-135)

**Dermal Minipig FMF:**

- 22) A 4-week dermal toxicity study in minipigs (T-51/203-166)
- 23) A 13-week dermal toxicity study in juvenile minipigs (T-52/203-191)
- 24) A 26-week dermal toxicity study in minipigs (T-53/203-190)

**Genetic Toxicology Studies:**

- 1) Mutagenicity test using salmonella typhimurium (T-119/203-018)
- 2) Mutagenicity test using salmonella typhimurium (T-120/203-215)
- 3) Mutation assay at the thymidine kinase locus of mouse lymphoma L5178Y cells (T-121/203-125)
- 4) Chromosomal aberration test with V79 Chinese hamster cells (T-123/203-021)
- 5) Mouse bone marrow micronucleus test by the oral route (T-124/203-111)

**Carcinogenicity Studies:**

Note: The photocarcinogenicity study conducted with 1% ASM 981 cream was reviewed under IND \_\_\_\_\_ and will be summarized in this review.

Note: The oral carcinogenicity studies conducted with ASM 981 were reviewed under IND \_\_\_\_\_ and will be summarized in this review.

Note: The dermal carcinogenicity studies conducted with 1% ASM 981 cream were reviewed in more detail in an addendum review to the NDA and will be summarized in this review.

- 1) SDZ ASM 981 cream: 52 week photocarcinogenesis study in hairless mice (T-128/BS-119)
- 2) Oncogenicity study by oral gavage administration to CD-1 mice for their life span (T-126/BS-32)
- 3) Oncogenicity study by oral gavage administration to \_\_\_\_\_ Wistar rats for 104 weeks (T-130/BS-381)
- 4) Oncogenicity study by oral gavage administration to \_\_\_\_\_ Wistar rats for 104 weeks (T-131/BS-358)
- 5) Carcinogenicity study by dermal administration to mice (T-127/BS-530)
- 6) 104-week dermal carcinogenicity study in rats (T-133/BS-733)

#### **Reproductive Toxicology Studies:**

- 1) An oral reproductive toxicity dose range finding study in female rats with toxicokinetics and placental transfer (Drug form: lyophilisate suspension) (T-111/203-091)
- 2) An oral combined fertility and embryo-fetal development study in rats (Drug form: lyophilisate suspension) (T-112/203-113)
- 3) An oral pre- and post-natal development in rats (Drug form: lyophilisate suspension) (T-113/203-160)
- 4) A dermal embryo-fetal development study in rats (Drug form: MF cream) (T-114/203-210)
- 5) Oral embryo-fetal development dose-range finding study in rabbits with toxicokinetic and placental transfer (Drug form: solid dispersion) (T-115/203-096)
- 6) An oral embryo-fetal development study in rabbits (T-116/203-140)
- 7) A dermal embryo-fetal development study in rabbits (Drug form: MF cream) (T-117/203-203)

#### **Special Toxicology Studies (with FMF):**

- 1) Primary eye irritation study in rabbits (T-73/203-163)
- 2) Determination of phototoxicity in guinea pigs (T-82/203-170)

**STUDIES NOT REVIEWED WITHIN THIS SUBMISSION:****Nonclinical Pharmacology Studies:****In Vitro Studies:**

- 1) Binding of SDZ ASM 981 to the FK506 binding protein FKBP-12 (macrophilin-12) (P-1/RD-2000-00563)
- 2) Interaction of SDZ ASM 981 (SDZ 280-981) complexed with FKBP on calcineurin activity in vitro (P-2/RD-2000-00632)
- 3) A novel anti-inflammatory drug, SDZ ASM 981, for the treatment of skin diseases: *in vitro* pharmacology (P-3/Journal article)
- 4) The activity of SDZ ASM 981 in human mixed lymphocyte reaction (P-4/PKF-97-01242)
- 5) In vitro activity of SDZ ASM 981 on the allergen-mediated stimulation of a human T cell clone (P-5/PKF-97-03400)
- 6) The effect of SDZ ASM 981 on the degranulation of murine mast cells stimulated with IgE and antigen (P-6/RD-2000-00698)
- 7) Effect of SDZ ASM 981, FK506 and cyclosporin A on IL-8 secretion in HaCaT keratinocytes, 1BR3GN fibroblasts and primary human umbilical vein endothelial cells (HUVEC) (P-7/RD-2000-01032)

**Animal Studies:**

- 8) Different effects of SDZ ASM 981 and FK 506 (Prograf<sup>®</sup>) on induction and elicitation phase of oxazolone-induced allergic contact dermatitis in mice (P-8/RD-2000-01052).
- 9) Effects of cyclosporine A on induction and elicitation of oxazolone-induced allergic contact dermatitis in mice (P-9/RD-1999-03347)
- 10) Oral SDZ ASM 981 is superior to FK 506 (Prograf<sup>®</sup>) against allergic contact dermatitis in rats (P-10/RD-2000-01249)
- 11) A novel anti-inflammatory drug, SDZ ASM 981, for the topical and oral treatment of skin disease: *in vivo* pharmacology (P-11/Journal article)
- 12) Topical activity against acute and sub-chronic irritant contact dermatitis (P-12/RD-2000-00429)
- 13) Oral ASM 981 inhibits magnesium deficiency-induced dermatosis of hairless rats (P-13/PKF-97-02993)
- 14) Comparison of the effects of SDZ ASM 981, Cyclosporin A and FK 506 on the localized Graft-vs.-Host reaction (P-14/RD-2000-01501)
- 15) Effect of ASM 981 and tacrolimus (FK 506) on allogeneic kidney transplantation (rat) (P-15/PKF-95-02729)

**Minor Amendment Pharmacology Studies:**

- 16) SDZ ASM 981: Penetration into and permeation through rat and pig skin in comparison to FK 506 (RD-2001-00635)
- 17) Effects of SDZ ASM 981, as compared to FK506 and cyclosporin A, in a rat model of graft-versus-host reaction (RD-2001-00237)

- 18) Effect of ADZ ASM 981 (SDZ 290-981) on IgM anti SRBC antibody formation (rat) (RD-2000-00336)

**Nonclinical Safety Pharmacology Studies:**

- 1) Effect of SDZ ASM 981 on lung function in the guinea-pig (safety pharmacology) (P-16/PKF-94-01016)
- 2) Effects on cardiovascular and respiratory systems in anaesthetized rats (P-17/784080)
- 3) Modified Irwin screen test in mice (P-18/784078)
- 4) Effect on endocrine function (P-19/784091)

**Acute Toxicology Studies:**

- 1) Acute oral toxicity study in mice (T-1/203-029)
- 2) Acute intravenous toxicity study in mice (T-2/203-028)
- 3) Acute oral toxicity study in rats (T-3/203-030)
- 4) Acute dermal toxicity study in rats (SDZ ASM 981 ——— ) (T-4/203-184)
- 5) Acute intravenous toxicity study in rats (T-5/203-027)

**Repeat Dose Toxicology Studies:**

**Oral Mice:**

- 1) A 13-day oral (per gavage) toxicity study in mice (T-8/BS-615) (Note: Only a summary of the study was provided in the submission instead of the full study report.)
- 2) A 2-week oral (per gavage) dose-range finding study in mice (drug form: lyophilisate suspension) (T-6/203-066)
- 3) A 2-week oral (per gavage) toxicokinetic study in mice (Drug form: solid dispersion) (T-9/203-156)

**Dermal Mice non-FMF:**

- 4) Toxicokinetic study by dermal administration to CD-1 mice for 2-weeks (Drug form: cream) (T-11/203-174)
- 5) 7-day dermal tolerability study in mice ——— 3944391; T-14/203-097)
- 6) Amendment No. 1 to report: 7-day dermal tolerability study in mice ( ——— 3944301; T-15/BS-38)
- 7) Toxicity to mice by dermal administration for 2 weeks ( ——— report no. SDZ 496/950759; T-16/203-064)
- 8) Preliminary toxicity study by dermal administration to CD-1 mice for 2 weeks ——— — no. 95/SPM059/0852; T-17/203-088)

**Oral Rats:**

- 9) A 2-week oral (gavage) dose-range-finding study in rats (Drug form: lyophilisate suspension) (T-21/203-089)

- 10) A 4-week oral (per gavage) toxicity study in rats (Drug form: lyophilisate suspension) (T-23/203-094)
- 11) A 4-week oral (per gavage) toxicity study in rats (Drug form: solid dispersion) (T-25/203-093)
- 12) A 4-week oral (per gavage) exploratory study in rats (T-93/203-159)
- 13) A 5-week oral (per gavage) exploratory study in rats with NMR examinations (T-94/203-185)

Dermal Rats FMF:

- 14) 2-week dermal dose-range finding study in rats (T-29/203-204)

Dermal Rats non-FMF:

- 15) (Formulation 1): Two-week dermal toxicity study in the rat — study lab no. 14918; T-33/203-138)
- 16) (Formulation 2): Two-week dermal toxicity study in the rat — study lab no. 15018; T-34/203-043)
- 17) (Formulation 3): Two-week dermal toxicity study in the rat — study lab no. 15019; T-35/203-116)
- 18) A 4-week dermal toxicity study in rats — study lab no. 15193; T-36/203-050)
- 19) Suppl. No. 1 to report: 4-week dermal toxicity study in rats {203-116} (T-37/203-051)
- 20) A 13 week dermal toxicity study in rats — study lab no. 15277; T-38/203-085)
- 21) Suppl. No. 1 to study: a 13-week dermal toxicity study in rats {203-085} (T-39/203-087)

Dermal Minipigs non-FMF:

- 22) SDZ ASM 981 (Formulation 1): 2-week dermal toxicity study in the minipig (T-55/203-114)
- 23) SDZ ASM 981 (Formulation 2): 2-week dermal toxicity study in minipig (T-56/203-044)
- 24) Suppl. No. 1 to study: Two-week dermal toxicity study in the minipig {203-044} (T-57/203-083)
- 25) SDZ AMS 981 (Formulation 2): Suppl. to study: Two-week dermal toxicity study in the minipig {203-044} (T-58/203-148)
- 26) SDZ ASM 981 (Formulation 3): Two-week dermal toxicity study in the minipig (T-59/203-115)
- 27) 3-week dermal toxicity study in minipigs (T-60/203-139)
- 28) 4-week dermal toxicity study in minipigs (T-61/203-048)
- 29) Suppl. no. 1 to study: 4-week dermal toxicity study in minipigs {203-048} (T-62/203-049)
- 30) Suppl. to study: 4 week dermal toxicity study in minipigs {203-018} (T-63/203-151)
- 31) 4-week dermal toxicity study in minipigs (T-64/203-145)
- 32) 4-week dermal toxicity study in minipigs (T-65/203-146)
- 33) 4-week dermal toxicity study in minipigs (T-66/203-143)
- 34) Amendment no. 1: SDZ ASM 981 — Formulation H: 4-week dermal toxicity study in minipigs {203-143} (T-67/203-144)
- 35) 4 week dermal toxicity study in minipigs (T-68/202-141)

- 36) Amendment no. 1: ADX SM 981 — Formulation J: 4-week dermal toxicity study in minipigs {202-141} (T-69/202-142)
- 37) A 13-week dermal toxicity study in minipigs (T-70/203-084)
- 38) Suppl. to study: A 13-week dermal toxicity study in minipigs {203-084} (T-71/203-152)
- 39) Determination of concentrations of SDZ ASM 981 in whole blood. Suppl. to study: A 13-week dermal toxicity study in minipigs {203-084} (T-72/203-134)

### **Special Toxicology Studies:**

#### **Special Toxicology Studies (with FMF):**

- 1) Investigation of skin irritative potential of 2 formulations using an in vitro human epidermis model — (T-87/BS-694)
- 2) Contact hypersensitivity in albino guinea pigs modified Buhler method (T-79/203/179)
- 3) Assessment of contact (photo)allergenic potential with the murine local lymph node assay (T-83/203-194)

#### **Ocular Irritation in Rabbits non-FMF**

- 1) Primary eye irritation study with — vehicle in rabbits (T-75/203-024)
- 2) Primary eye irritation study with — 0.3% in rabbits (T-76/203-025)
- 3) Primary eye irritation study with — 0.1% in rabbits (T-77/203-026)
- 4) Acute eye irritation study in rabbits (T-78/BS-689)

#### **Skin Sensitization/Contact Hypersensitivity in Guinea Pigs non-FMF**

- 5) Skin sensitization study in guinea pig (T-80/203-020)
- 6) Delayed contact hypersensitivity study in guinea pig (T-81/203-098)

#### **Photoallergenic Potential (LLNA) in Mice non-FMF:**

- 7) Assessment of immunosuppressive potential during contact allergy. A dose range finding study using the murine local lymph node assay (T-84/BS-614)
- 8) Assessment of immunosuppressive potential during contact allergy. A comparison with ASM981 and FK506 using the murine local lymph node assay (T-85/BS-621)
- 9) Assessment of contact (photo)allergenic potential with the murine local lymph node assay (T-86/BS-30)

#### **Antigenicity non-FMF**

- 10) Antigenicity study of SDZ ASM 981 (T-88/BS-716)

**By-products/Impurities Studies:****Pharmacology:**

- 1) By-products of SDZ ASM 981 synthesis are less potent inhibitors of the antigen-specific proliferation of human helper T-cells (T-109/RD-2000-01009)
- 2) By-products of SDZ ASM 981 synthesis inhibits murine allergic contact dermatitis (T-110/RD-2000-01245)

**Oral Toxicity Rats:**

- 3) Toxicity study by oral gavage administration to ——— Wistar rats for 2 weeks (for ASM 981 spiked with 4.9% 1-96) (T-91/203-161)
- 4) 2-week oral (gavage) toxicity study in rats (for ASM 981 spiked with 9.1% 514-95) (T-98/203-202)
- 5) 2-week oral toxicity study in rats (for ASM 981 spiked with 5.1% 2-97) (T-99/203-214)

**Dermal Toxicity Rats FMF:**

- 6) 2-week dermal toxicity study in rats (for heat stressed ASM 981 cream) (T-100/203-208)

**Mutagenicity:**

- 7) Mutagenicity test using salmonella typhimurium (for ASM 981 spiked with 5% 1-96) (T-101/203-157)
- 8) Mutagenicity test using Salmonella typhimurium (for ASM 981 spiked with 9% 514-95) (T-102/202-183)
- 9) Mutagenicity test using Salmonella typhimurium (for ASM 981 spiked with 4.2% 515-95, 5.3% 516-95 and 5.6% 522-94) (T-103/BS-167)
- 10) Mutagenicity test using Salmonella typhimurium (for ASM 981 spiked with 4.9% 2-97) (T-104/BS-31)
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- 12) Chromosomal aberration test with V79 Chinese hamster cells (for ASM 981 spiked with 5% 2-97) (T-107/203-199)
- 13) Chromosomal aberration test with V79 Chinese hamster cells (for ASM 981 spiked with 4.2% 515-95, 5.3% 516-95 and 5.6% 522-94) (T-108/BS-148)

**INTRODUCTION AND DRUG HISTORY:**

Pimecrolimus (ASM 981) is an ascomycin macrolactam derivative. It has been demonstrated that pimecrolimus is an inhibitor of inflammatory cytokine release. In addition, pimecrolimus possess immunosuppressant activity. A topical formulation of pimecrolimus, Elidel™ (1% ASM 981) cream, has been developed by Novartis for dermatologic use. Elidel™ cream is indicated for the treatment of atopic dermatitis.

The sponsor submitted IND — to the division in October 1997 for studying the efficacy and safety of 1% ASM 981 cream in the treatment of atopic dermatitis. The sponsor conducted many nonclinical pharmacology/toxicology studies under IND — during the development of the 1% ASM 981 cream. Many of the nonclinical topical toxicity studies were conducted with topical cream formulations for ASM 981 that differed from the final to be marketed 1% ASM 981 cream (Elidel™). All of the nonclinical studies conducted for ASM 981 cream will be listed in this NDA review. However, in general only the nonclinical studies that have been conducted with the final to be marketed 1% ASM 981 cream formulation will be reviewed here. A few of the studies that were conducted with other ASM 981 formulations may be mentioned in the review if they provide additional information beyond what is available from the nonclinical studies performed with the final to be marketed 1% ASM 981 cream formulation. A list of the studies that were and were not reviewed for this NDA were provided in the previous two sections of this review.

It is important to note that a Pharmacology minor amendment was submitted to the NDA on 6-8-01. This amendment contained final study reports for 3 new nonclinical pharmacology studies. The sponsor states that these nonclinical study reports were submitted to support statements in revised labeling that were to be submitted to the NDA within 2 weeks of the date of this submission. The results of the 3 nonclinical study reports are summarized in the Pharmacology summary section of the NDA review. A labeling supplement that contained revised draft labeling (package insert) was submitted on 6-21-01. The review of the label provided previously in this review used the electronic copy of the label included in the 6-21-01 labeling supplement submission.

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## PHARMACOLOGY:



### Pharmacology Summary:

ASM 981 binds to macrophilin-12 and inhibits the Ca-dependent phosphatase calcineurin. As a consequence, it inhibits T-cell activation by blocking the transcription of early cytokines. ASM 981 inhibited transcription of a reporter gene under the control of the IL-2 promoter in stably transfected Jurkat cells at a subnanomolar concentration ( $IC_{50} = 0.04$  nM). ASM 981 had a lower  $IC_{50}$  value compared to Cyclosporine by a factor  $> 10$  in this model.

The inhibitory effects on activation of T-cells *in vitro* were determined in a two-way mixed lymphocyte reaction. In this model, allogeneic major histocompatibility complex antigens induce differentiation of naïve T-cells into activated T-cells. ASM 981 inhibited the mixed lymphocyte reaction at nanomolar concentration (mouse and human  $IC_{50} = 1.3$  nM). ASM 981 had a lower  $IC_{50}$  value compared to Cyclosporine by a factor of  $> 10$  in this model.

ASM 981 inhibited proliferation and cytokine production of a human T-cell CFTS4:3.1 clone isolated from skin of an atopic dermatitis patient at nanomolar and subnanomolar concentrations, respectively. ASM 981 downregulated, at nanomolar concentrations, both Th1 (IL-2, INF- $\gamma$ ) and Th2 (IL-4, IL-5, IL-10) - type cytokine synthesis after antigen-specific activation in this model. ASM 981 had a lower  $IC_{50}$  value compared to Cyclosporine by a factor of  $\sim 10$  in this model. ASM 981 inhibited the IgE mediated activation of murine mast cells and the release of proinflammatory mediators ( $IC_{50} = 30$  nM). ASM 981 was approximately equipotent as Cyclosporine ( $IC_{50} = 53$  nM) in this model.

The effects of ASM 981, cyclosporine and FK506 on cell growth were investigated in HaCaT keratinocytes, 1BR3GN fibroblasts and primary human umbilical vein endothelial cells (HUVEC). Inhibitory effects on cell growth were seen with all three compounds only at concentrations above 1  $\mu$ M. This result indicates that none of the compounds appear to have a direct effect on inhibition of cell proliferation at concentrations that would have pharmacological significance (nM concentrations).

Several pharmacology studies were conducted in animal models of allergic contact dermatitis. Allergic contact dermatitis in mice, rats and pigs were used as models of T-cell mediated skin disease. ASM 981 exhibited anti-inflammatory activity in these animal models of skin inflammation after both topical and systemic administration. Murine allergic contact dermatitis was inhibited by topical ASM 981 and dexamethasone with comparable efficacy. Topical ASM 981 was as effective as the corticosteroids clobetasol-17-propionate and fluticasone in the pig model of allergic contact dermatitis. The activity of a cream formulation with 0.1% ASM 981 compared well with the activities of commercially available corticosteroid creams in the pig model of allergic contact dermatitis. Unlike clobetasol, topical ASM 981 did not cause skin atrophy in pigs. ASM 981 was also topically active in murine irritant contact dermatitis models. Oral ASM 981 reduced skin inflammation and pruritus in hypomagnesemic hairless rats, a model that mimics clinical aspects of atopic dermatitis. Oral treatment of allergic contact dermatitis in mice and rats revealed that ASM 981, FK506 and Cyclosporine were able to partially alleviate symptoms displayed in both animal models.

The systemic immunosuppressive activity of ASM 981 was investigated in two studies. The localized graft vs host reaction is a rat model for assessment of immunosuppression. The activity of ASM 981, cyclosporine and FK506 were compared in this model. The localized graft vs host reaction was inhibited in rats by subcutaneous administration of FK506 and Cyclosporine in a dose dependent manner. The calculated ED<sub>50</sub>s were < 0.1 mg/kg for FK506 and 1 mg/kg for Cyclosporine. No significant effect was observed after subcutaneous administration of ASM 981 up to a dose of 9 mg/kg. In summary, subcutaneous injections of nine times the effective dose of Cyclosporine and ninety times the effective dose of FK506 did not inhibit the localized graft-versus-host reaction in rats.

Allogeneic kidney transplantation was another animal rat model used for the assessment of immunosuppression. ASM 981 was compared with Cyclosporine and FK506 in this model. The lowest daily oral dose (14 days of treatment) at which all animals survived orthotopic allogeneic kidney transplantation for 100 days was 15.6 mg/kg ASM 981, 5.0 mg/kg Cyclosporine and 1.0 mg/kg FK506. ASM 981 had a lower potency to prevent kidney rejection compared to Cyclosporine and FK506 by a factor of 3 and 15, respectively. The results of this experiment suggest that ASM 981 is a less potent systemic immunosuppressive agent compared to Cyclosporine and FK506 in this rat model.

The three additional nonclinical pharmacology studies submitted in the pharmacology minor amendment (6-8-01) provided additional information on the immune suppressive activity of ASM 981 in various *in vivo* animal models and compared the *in vitro* permeability of ASM 981, FK506 and cyclosporine A in rat and minipig skin. One of the *in vivo* nonclinical pharmacology studies demonstrated that in the rat model of graft versus host reaction, Cyclosporine A and FK506 had an 8 and 66 fold greater activity than ASM 981. The other *in vivo* nonclinical pharmacology study showed that ASM 981 had ~50 fold lower immune suppressive activity in sheep red blood cell plaque forming cell assay compared to FK506. It is important to note that it is unclear how having less immune suppressive activity in both of these *in vivo* animal models after subcutaneous administration relates to the immune suppressive activity of topical administration of ASM 981 in the treatment of atopic dermatitis in humans. It is possible that ASM 981 may have greater immune suppressive activity than either FK506 or

cyclosporine A after topical administration in atopic dermatitis patients that have a compromised skin barrier function. In addition, although the *in vitro* pharmacology study demonstrated that ASM 981 showed lower permeation through rat and minipig skin compared to FK506, this may not be relevant in atopic dermatitis patients that have a compromised skin barrier function. Also, it is important to note that the vehicle used in this *in vitro* study is not the same as the marketed topical formulation for FK506 (Protopic) or of the to be marketed topical formulation of ASM 981 (Elidel).

**Pharmacology conclusions:**

The results of the *in vitro* and *in vivo* pharmacology studies support the pharmacological rationale that ASM 981 may be an effective drug to combat T-cell mediated inflammatory skin diseases. In addition, ASM 981 demonstrated a lower potential for affecting systemic immune responses compared to cyclosporine and FK506 in the two tested animal models.

The two additional nonclinical pharmacology studies submitted in the pharmacology minor amendment (6-8-01) supported that ASM 981 demonstrated a lower potential for affecting systemic immune responses compared to cyclosporine and FK506. Also, the *in vitro* nonclinical pharmacology study showed that ASM 981 had lower permeation thorough rat and minipig skin compared to FK506. The sponsor stated in the pharmacology minor amendment that these 3 nonclinical pharmacology study reports were submitted to the NDA to support statements in revised labeling to be submitted to the NDA within the next 2 weeks.

It will be recommended that these statements not be included in the label because they could be misleading. It is unclear how effects on systemic immune suppression in *in vivo* animal models after subcutaneous administration would compare to possible immune suppression potential of topically applied ASM 981 in atopic dermatitis patients that have a compromised skin barrier. Also, the lower permeation of ASM 981 *in vitro* through rat and minipig skin compared to FK506 may not be relevant in atopic dermatitis patients that have a compromised skin barrier function.

**SAFETY PHARMACOLOGY:**

**Safety pharmacology summary:**

The effects of ASM 981 on basal airway resistance and dynamic compliance were tested in anesthetized ventilated guinea pigs. No biologically relevant compound related effects on either parameter were noted up to the highest dose of 10 mg/kg iv. Airways reactivity to histamine was also not affected after injection of ASM 981 (up to 10 mg/kg iv).

Cardiovascular and respiratory effects of ASM 981 were investigated in anesthetized rats. No overt effects on ECG, systolic, diastolic and mean blood pressure, or on respiratory rate, tidal and minute volumes were noted up to the highest single oral dose of 90 mg/kg.

The effects of ASM 981 on the behavioral and physiologic state of mice were assessed in the modified Irwin test. No overt effects on the central nervous system were noted following a single oral dose up to 270 mg/kg.

The effects of ASM 981 on different endocrine parameters, e.g. the release of hormones of the pituitary, adrenals and testes as well as serum levels of glucose and calcium were investigated in male rats. No treatment related effects were noted up to the highest single oral dose of 40 mg/kg.

**PHARMACOKINETICS/TOXICOKINETICS:**

Note: Most of the ADME studies were conducted using <sup>3</sup>H labeled ASM 981. Radiolabeled ASM 981 was synthesized by the Isotope Laboratory, Novartis Pharma AG, Basel, Switzerland. Labeling with tritium was favored against a <sup>14</sup>C label, since the chemical introduction of a <sup>3</sup>H-label into the ASM 981 molecule was accomplished in \_\_\_\_\_

\_\_\_\_\_ The sponsor states that a higher specific radioactivity can be reached with tritium compared to labeling with <sup>14</sup>C label. The sponsor states that <sup>3</sup>H-label was metabolically stable as indicated by complete recovery in mass balance studies in rats after intravenous administration. The position of the isotopes in the <sup>3</sup>H-labeled ASM 981 in positions 5 and 6 of the piperidine ring.

A figure detailing the possible sites of biotransformation of ASM 981 by mouse, rat, rabbit, minipig and human liver microsomes and tautomerizations is provided in the review of Pharmacokinetic study #16.

**Mouse ADME:****Pharmacokinetic Study #1:**

*Absorption and disposition of [<sup>3</sup>H]ASM981 in mouse following a single oral dose of 15 mg/kg and comparison with a single intravenous reference dose of 3 mg/kg*

**Study Title:** Absorption and disposition of [<sup>3</sup>H]ASM981 in mouse following a single oral dose of 15 mg/kg and comparison with a single intravenous reference dose of 3 mg/kg

**Study No:** M-7/R98-491

Conducting laboratory: Novartis Pharma AG, Basel, Switzerland  
Study release date: July 21, 2000  
GLP compliance: No

The objective of this study was to investigate the absorption and disposition of ASM 981 in male CD-1 mice. The report states that this study was conducted to support the mouse oncogenicity study with information for a comparison of the metabolism of ASM 981 in mouse and man. [<sup>3</sup>H] ASM 981 was administered as a single oral dose of 15 mg/kg (corresponding to the mid dose of the oncogenicity study) and as a single intravenous dose of 3 mg/kg as a reference.

Blood samples were collected at 0.083 (iv only), 0.5, 1, 3, 8, 24, 72 and 168 hours post dose. Urine, feces and cage washes were collected separately and individually over the time intervals 0-24, 24-48 and 48-72 hours post dose. Samples were obtained from 3 mice/timepoint. Radioactivity in the administration ASM 981 suspensions, blood, urine, feces, carcasses and cage washes were measure by \_\_\_\_\_ Metabolite profiles from blood, urine, and feces were determined by HPLC.

Mean pharmacokinetic parameters for total radioactivity and for the parent compound following a single oral dose of 15 mg/kg or a single intravenous dose of 3 mg/kg [<sup>3</sup>H] ASM 981 are provided in the following table.

Measurement	C <sub>max</sub> (pmol/ml)		T <sub>max</sub> (hours)		AUC (pmol·hr/ml)	
	po	iv	po	iv	po	iv
Radioactivity	1001	3310	0.5	--	18933	10182
ASM 981	246	3082	0.5	--	1184	3397

The estimated bioavailability after oral dosing was calculated as 7%. The parent drug (ASM 981) accounted for 33% of the total radioactive AUC after iv dosing and 6.3% after oral dosing. This result indicates that ASM 981 underwent extensive metabolism after administration (especially after oral dosing).

It was demonstrated in this study that the tritium label in ASM 981 was metabolically stable. Approximately 0.5% of the dose of radioactivity was converted to tritiated water after oral dosing and ~0.2% after iv dosing.

The metabolite patterns in blood were highly complex. — peaks for [<sup>3</sup>H]ASM 981 tautomers were present. A large number of only partially separated metabolites of medium polarity and a front peak (designated —, was noted in the HPLC profile. Metabolite peaks were named according to there approximate retention times. No attempt was made to characterize the structures of the numerous metabolites in this study. The study report states that the relative abundance of the metabolites of medium polarity was similar to that found in the rat but higher than in the minipig and man. The results of these separate studies will be described below. The relative contribution of the metabolites of medium polarity to the AUC of radioactivity was considerably larger after oral than after iv dosing. The metabolites and parent

drug areas under the curve in blood after oral (15 mg/kg) and iv (3 mg/kg) dosing is provided in the following table.

Component/ Peak	Oral AUC <sub>(0-72 hr)</sub>		Intravenous AUC <sub>(0-72 hr)</sub>	
	pmol·hr/ml	%	pmol·hr/ml	%
—	1844	14.0	1191	14.8
—	441	3.4	205	2.6
—	279	2.1	89	1.1
—	368	2.8	129	1.6
—	180	1.4	43	0.5
—	257	2.0	74	0.9
—	204	1.6	147	1.8
—	236	1.8	127	1.6
ASM 981 <sup>a</sup>	1181	9.0	3365	41.9
Residual components <sup>b</sup>	3475	26.5	1294	16.1
Total Detected	8465	64.5	6665	83.0
Lost during sample processing	4669	35.5	1368	17.0
Total in original sample	13134	100.0	8033	100.0

a – sum of major and minor tautomer

b – minor metabolites not separated chromatographically

The small percentage of the dose of radioactivity excreted in urine (~2% after both oral and iv administration) was composed of mainly or exclusively of highly polar material. The metabolite patterns in the feces were extremely complex, showing mainly a broad hump of chromatographically unresolved components centered at 55 min retention time. No parent drug was found in the feces after iv dosing. This suggests that no biliary excretion of unchanged ASM 981 occurred after iv dosing. It appears that ASM 981 is eliminated in the mouse exclusively by metabolism. Approximately 3% of the dose appeared in the feces as unchanged ASM 981, which represented unabsorbed compound.

The study report states that the complexity of the metabolite patterns has probably two reasons. First, the occurrence of a large number of parallel and consecutive biotransformation reactions. Second, the existence of multiple, slowly interconverting tautomers of most metabolites. Such tautomers also exist for the parent drug.

The bulk of the radioactivity was recovered in the feces after oral and iv dosing (means of 76.2 and 87.8% of the dose at 24 and 168 hours after oral dosing and means of 85.8 and 95.4% at 24 and 168 hours after iv dosing, respectively). Residual radioactivity in the carcasses at 168 hours post dose amounted to less than 0.8% of the dose after oral and iv dosing. The results after iv dosing suggest that the excretion occurred predominantly via the bile. The excretion into the urine was marginal (2.2% of the oral dose and 1.8% of the iv dose recovered in urine within 168 hours post dose). The material balance of radioactivity (including the cage washes and carcasses) was essentially complete after 168 hours (93.4% and 99.3% of the dose after oral and iv dosing, respectively).



Measure	C <sub>max</sub> (pmol/ml)			T <sub>max</sub> (hours)			AUC (pmol·hr/ml)		
	iv	Low po	High po	iv	Low po	High po	iv	Low po	High po
Radio-activity	--	195 ± 92	4478 ± 2804	--	2.5 ± 1.0	4.8 ± 2.9	1259 ± 227	3470 ± 697	116263 ± 69727
ASM 981	--	183 ± 154	2071 ± 1422	--	1.4 ± 0.8	2.3 ± 2.0	374 ± 30	512 ± 478	27746 ± 22114

The total absorption following low and high oral doses of [<sup>3</sup>H] ASM 981 was 55 ± 11% and 93 ± 55%, respectively. The estimated bioavailability appeared to be higher after the high oral dose (74 ± 59%) than after the low oral dose (27 ± 26%). The study report states that this may be explained by the non-linearity of the first pass effect. The parent drug (ASM 981) accounted for 29% of the total radioactive AUC after the iv dose, 15% after low oral dose and 24% after high oral dose. This result indicates that ASM 981 underwent extensive metabolism after administration.

The permeation of radioactivity was low (~5% of the dose) after multiple topical administration of ASM 981 (3 mg/kg/day of Formulation B, 0.03%, for 5 days). The estimated radioactivity concentration at the site of application (after stripping the stratum corneum) was 1750 pmol/g at 72 hour after the last topical administration. Parent drug represented almost half of the radioactivity in the skin at the site of application at 72 hours after the last application.

The orally absorbed material distributed extensively into the tissues. Mainly into the liver, adrenals, blood vessel wall and gastro-intestinal tract. These tissues may be potential targets organs of toxicity based on higher levels of distribution of the radioactivity.

Metabolite patterns were determined in blood after the intravenous and two oral doses. The blood levels of radioactivity were too low for radiochromatography following topical administration. The radiochromatograms of pooled blood extracts showed essentially a — peak for ASM 981 (tautomers), a large number of mostly very minor metabolites eluting between 45 and 100 minutes, and a front peak —. The peaks in the middle region of the chromatograms were too numerous to be completely separated and only three of them — were abundant enough or sufficiently separated to be observed consistently. — was very minor, — and — were seen in slightly higher concentrations. — was more prominent in the animals of the high oral dose group.

The metabolites and parent drug areas under the curve in blood after intravenous (1 mg/kg), low oral (5 mg/kg) and high oral (100 mg/kg) dosing is provided in the following table.

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Peak	Intravenous AUC <sub>(0-24 hr)</sub>		Low Oral AUC <sub>(0-48 hr)</sub>		High Oral AUC <sub>(0-48 hr)</sub>	
	nmol·hr/ml	%	nmol·hr/ml	%	nmol·hr/ml	%
—	0.129	12	0.602	26	10.2	13
—	0.006	1	0.006	0.3	2.3	3
—	0.076	7	0.085	4	4.7	6
—	0.032	3	0.041	2	8.4	11
ASM 981 <sup>a</sup>	0.367	35	0.581	25	19.4	25
Residual <sup>b</sup>	0.431	41	1.042	44	33.6	43

a – sum of major and minor tautomer

b – minor metabolites not separated chromatographically

Only ~3% of the radioactivity administered was excreted into urine following intravenous or oral administration. The radiochromatogram consisted mainly of — peaks at the front. Part of this polar material represented tritiated water. No parent drug was detected in urine. The metabolite patterns in feces extracts were complex. The radiochromatogram showed a broad hump between 30 and 80 min retention time, probably composed of a large number of minor peaks, and a front peak, representing one or several very polar metabolites. Total percent of radiochromatogram contributed to metabolites was 75%, 81% and 41% after iv, low oral and high oral dose administration, respectively. Parent drug was not detected in feces after iv administration but was detected in feces after oral administration. Total percent of radiochromatogram contributed to ASM 981 was <0.1%, 7% and 32% after iv, low oral and high oral dose administration, respectively. The ASM 981 detected in feces was due to unabsorbed ASM 981.

Drug related material was excreted almost exclusively in the feces independent of the route of administration. The mean fecal and urinary excretion was  $72.1 \pm 17.1$  and  $2.6 \pm 0.6\%$  of the dose, respectively, following iv administration. The fraction of the dose recovered in the carcass after iv administration was  $24.1 \pm 16.0\%$ . The contract lab explained this unexpected high percentage as related to the fact that the intestinal contents were not separated from the carcass for radioactivity determination after iv administration. A mean of  $88.1 \pm 4.5$  and  $3.4 \pm 1.1\%$  of the dose was excreted in feces and urine, respectively, after low oral dose administration. The residual radioactivity in the carcass was  $3.3 \pm 1.6\%$ . A mean of  $72.4 \pm 8.8$  and  $3.4 \pm 2.1\%$  of the dose was excreted in feces and urine, respectively, after high oral dose administration. The residual radioactivity in the carcass was  $2.0 \pm 2.5\%$ . After multiple topical applications,  $3.4 \pm 1.0\%$  and  $0.3 \pm 0.2\%$  of the total dose were recovered in the feces and urine, respectively. The fraction of the dose recovered in the carcass, excluding the skin at the application site, at the end of the experimental period was  $0.3 \pm 0.2\%$ . The recovery of radioactivity was complete in the iv, low oral and multiple topical studies (mean  $\geq 93\%$ ). The recovery of radioactivity was  $77.7 \pm 9.3\%$  in the high oral dose study.

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ON ORIGINAL

**Pharmacokinetic Study #3:**

*Supplementary metabolism study in the rat with administration of a single oral dose of 100 mg/kg [<sup>3</sup>H]ASM981*

Study Title: Supplementary metabolism study in the rat with administration of a single oral dose of 100 mg/kg [<sup>3</sup>H]ASM981  
Study No: M-9/R98-444  
Conducting laboratory: Novartis Pharma AG, Basel, Switzerland  
Study release date: July 28, 2000  
GLP compliance: No

The metabolism of ASM 981 in animals and man is highly complex. Therefore, it has been determined that \_\_\_\_\_ is needed, in addition to radiochromatography, for comparing the metabolites in different species. The objective of the present study was to validate the *in vitro* approach by comparing the metabolites found *in vitro* (will be discussed in detail in latter pharmacokinetic studies) with those formed *in vivo* under conditions allowing their structural characterization by \_\_\_\_\_ using the rat as the test species. The *in vitro* studies were conducted in liver microsomes of various toxicological test species and man to be able to obtain sufficiently high amounts of metabolites to obtain informative \_\_\_\_\_ analysis. Three male Wistar rats were treated with a single oral dose of 100 mg/kg [<sup>3</sup>H] ASM 981 and sacrificed five hours later (approximate T<sub>max</sub> of radioactivity). Blood and a number of tissues (liver, kidney, lung, dorsal skin, mesenteric lymph nodes and whole brain) were obtained for analysis. The tissues obtained were selected on the basis of their toxicological and pharmacological relevance for ASM 981. Extracts of the samples were analyzed by HPLC \_\_\_\_\_ and by \_\_\_\_\_

The metabolites in the blood and tissues, which could be characterized by \_\_\_\_\_, were all products of either demethylations or oxygenations. The oxygenations were demonstrated in the *in vitro* studies to be hydroxylations. The data showed that primary metabolites predominated in the blood and in all the tissues, except for the liver. The primary metabolites resulted from a hydroxylation and two different O-demethylations. Several secondary metabolites were noted in the liver extracts. The study report states that the metabolite profile obtained from the liver extract was similar to the metabolite profile obtained from the rat liver microsomes after longer incubation times.

For the three main primary metabolites, the following structural details were derived from the \_\_\_\_\_. The hydroxylation metabolite (designated Ox\_7) is formed by hydroxylation of a CH<sub>3</sub> group in the lower half of the molecule which undergoes further hydroxylation to a carboxylic acid (in rabbit liver microsomes). The first demethylation metabolite (designated Dy\_6) is the product of an O-demethylation at the methoxy group in the major side-chain in the upper half of the molecule. The second demethylation metabolite (designated Dx\_1) results from an O-demethylation near position 23 or 25 of the macrocycle.

The concentrations (pmol/g) of ASM 981 and the three primary metabolites in blood and tissues are provided in the following table.

Peak	Blood	Liver	Kidney	Lung	Dorsal Skin	Mes. lymph nodes	Whole Brain
↖ (front peak)	77	287	294	86	136	85	16
Dx 1	62	ng	297	252	98	308	10
Dy 6	41	596	443	417	213	666	30
Ox 7	52	1248	882	807	485	nd	32
ASM 981 <sup>a</sup>	137	1266	1969	2510	1929	4475	226
Additional Components <sup>b</sup>	608	13120	2497	1929	861	2454	48
Total Detected	978	16518	6381	6002	3718	7987	362

a – sum of major and minor tautomer

b –metabolites not identified by ↖ or not separated chromatographically

The total concentration of drug-related radiolabeled material at 5 hours postdose in the organs analyzed ranked in the following order: liver > mesenteric lymph nodes > kidney > lung > dorsal skin > blood > whole brain. ASM 981 was the most abundant radiolabeled component in the blood and in all the tissues examined in this study. Only 1.5% of the dose was converted to tritiated water.

#### Pharmacokinetic Study #4:

\_\_\_\_\_ in albino and pigmented rats after po and iv administration of [<sup>3</sup>H]ASM981

Study Title: \_\_\_\_\_ in albino and pigmented rats after po and iv administration of [<sup>3</sup>H]ASM981

Study No: M-10/303-200

Conducting laboratory: Novartis Pharma AG, Basel, Switzerland

Study release date: July 17, 1998

GLP compliance: No

The objective of this study was to determine the affinity of ASM 981 and/or its metabolites to melanin in the rat. The affinity of ASM 981 and/or its metabolites to melanin in the pigment rat was investigated by \_\_\_\_\_ following 1 mg/kg iv and 5 mg/kg oral doses of [<sup>3</sup>H] ASM 981. The concentrations of radioactivity in the melanin containing structures of pigmented rats (Long Evans rats) was compared to those observed in albino rats (Wistar rats).

Four pigmented rats (one per time point and dosing route) were sacrificed at 5 min and at 169 hr after iv injection and 2 hr and 168 hr after oral gavage. Two albino rats were sacrificed at 5 min after iv injection and 2 hr after oral gavage. After sacrifice, rats were frozen, sagittal sections of 40 μm thickness were obtained of the whole rat and the level of radioactivity was assessed via ↖ for each sagittal section. Similar concentrations of radioactivity were noted in the sclera and/or chorioidea in pigmented and albino rats at 5 min after iv dosing. Low

concentrations of radioactive material was noted in the uveal tract of the eye in pigmented rats at 168 hours after iv dosing and at 2 and 168 hours after oral dosing. These results indicate that ASM 981 and/or its metabolites do not have a specific affinity to melanin in the rat under the conditions of this study.

#### Pharmacokinetic Study #5:

*Embryo-fetal transfer in pregnant rats on day 13 and on day 17 of gestation after po administration of [<sup>3</sup>H]ASM981*

Study Title: Embryo-fetal transfer in pregnant rats on day 13 and on day 17 of gestation after po administration of [<sup>3</sup>H]ASM981  
Study No: M-11/303-201  
Conducting laboratory: Novartis Pharma AG, Basel, Switzerland  
Study release date: July 17, 1998  
GLP compliance: No

The objective of this study was to determine the embryo-fetal transfer of ASM 981 and/or its metabolites into the fetal compartment following a 45 mg/kg oral dose of [<sup>3</sup>H] ASM 981 to pregnant rats on day 13 and day 17 of gestation. The 45 mg/kg oral dose of ASM 981 corresponds to the high dose group of the embryo-fetal development study in rats. Four pregnant female rats were administered ASM 981 via gavage on day 13 to assess the embryo-fetal transfer of ASM 981 and/or its metabolites at the end of organogenesis. Radioactivity was determined by \_\_\_\_\_ in the organs/tissues of these rats. Six pregnant female rats were administered ASM 981 via gavage on day 17 to examine the levels of ASM 981 and/or its metabolites towards the end of the maturation phase. Four rats were processed by \_\_\_\_\_ and two were forwarded to another division for biotransformation work. The biotransformation results will be reported in the next study.

From the animals administered ASM 981 on day 13 of gestation, the major maternal organs (adrenals, blood, plasma, blood vessel wall, bone marrow, brain, heart, kidney, large intestine, liver, lung, lymph nodes, muscle, pancreas, salivary gland, skin, spleen, thymus, thyroid and white fat), the embryos and placentas were dissected out and processed by \_\_\_\_\_ at 0.5, 2, 6 and 24 hours post dose (one rat with corresponding embryos and placentas per time point). The pregnant animals treated on day 17 of gestation were sacrificed at the same time points as above and the radioactivity concentrations in maternal and fetal tissues, placentas, amnion and amniotic fluid were determined by \_\_\_\_\_

The overall concentrations of radioactive substances in the maternal tissues on day 13 and day 17 of gestation were higher than those in maternal blood at all the time points investigated and maximum at 2 hours post dose in most of the tissues. The highest concentrations of radioactivity were observed in the liver and adrenal. The fetuses were exposed to the compound and/or its metabolites. The maximal radioactivity concentrations in the fetuses (1.2 nmol/g at 24 hour post dose on day 13 and 0.6 nmol/g at 6 hours post dose on day 17 of gestation) were similar to those in maternal blood and much lower than the maternal tissue concentrations at all time points. Overall, the data suggest that following a 45 mg/kg oral dose of ASM 981 during

day 6 to day 18 of gestation in rats, the fetuses were exposed to ASM 981 and/or its metabolites during the whole treatment period.

**Pharmacokinetic Study #6:**

*Embryo-fetal transfer in pregnant rats on day 13 and day 17 of gestation after po administration of [<sup>3</sup>H]ASM981. Supplementary biotransformation data*

**Study Title:** Embryo-fetal transfer in pregnant rats on day 13 and day 17 of gestation after po administration of [<sup>3</sup>H]ASM981. Supplementary biotransformation data  
**Study No:** M-12/R98-375-01  
**Conducting laboratory:** Novartis Pharma AG, Basel, Switzerland  
**Study release date:** July 18, 2000  
**GLP compliance:** No

The objective of this study was to determine the metabolite patterns in blood and fetuses from the two designated pregnant Wistar rats mentioned in the previous study. These rats received a 45 mg/kg oral ASM 981 dose on day 17 of gestation. The following samples were analyzed for metabolite patterns. The blood from one of the treated pregnant rats 2 hours after dose administration. The pool of fetuses from the other treated pregnant rat 2 hours after dose administration. Blood samples and fetal tissue homogenates were analyzed for the metabolite pattern by HPLC

Parent drug was the most abundant radiolabeled component in both the blood and the fetuses at 2 hours post dose. It accounted for about 20% of the total radioactivity in the blood and about 50% of the radioactivity in the fetuses. The remaining radioactivity in the blood was due to numerous, mostly minor metabolites, which were only partially separated by chromatography. Mainly the most nonpolar metabolites were also found in the fetuses. The absolute concentration of ASM 981 in the fetuses was about half that in the blood. The absolute concentration of total metabolites in the fetuses was ~10% of that in the blood.

**Rabbit ADME:****Pharmacokinetic Study #7:**

*Placental transfer in rabbits after peroral administration of 20 mg/kg of [<sup>3</sup>H]ASM981*

**Study Title:** Placental transfer in rabbits after peroral administration of 20 mg/kg of [<sup>3</sup>H]ASM981  
**Study No:** M-13/R98-494  
**Conducting laboratory:** Novartis Pharma AG, Basel, Switzerland  
**Study release date:** July 10, 2000  
**GLP compliance:** No

The objective of this study was to determine the placental transfer of ASM 981 and/or metabolites in pregnant rabbits. Pregnant New Zealand White rabbits were administered an oral dose (gavage) of 20 mg/kg [<sup>3</sup>H]ASM 981 on gestational day 17. Maternal blood and plasma samples were obtained at 0.25, 0.5, 1, 2, 4, 8 and 24 hours post dose. Maternal urine was obtained over the period of 0 – 24 hours post dose. Fetuses, amniotic fluid and placentas were obtained at 24 hours for analysis. The level of radioactivity was determined in maternal blood, plasma and urine and fetuses, amniotic fluid and placentas by \_\_\_\_\_ The metabolite profiles in maternal blood and urine and fetuses was determined by HPLC —

The mean radioactivity concentrations in the plasma, fetuses, placentas and amniotic fluids, determined at 24 hours post dose, were very similar (i.e., 0.75, 0.85, 0.66 and 0.83 nmol/g, respectively). This result suggests that an even distribution of ASM 981 and/or metabolites throughout the embryo-fetal compartment.

The average radioactivity concentrations in blood and plasma increased over the measurement period (15 minutes to 24 hours) from 0.07 (blood) or 0.14 μmol/L (plasma) to 0.75 μmol/L (both blood and plasma). The onset of absorption was slow since virtually no radioactivity was observed in blood and plasma up to and including 0.5 hr. The metabolite patterns revealed average blood concentrations of ASM 981 of 0.008, 0.009 and 0.005 μmol/L at 1, 8 and 24 hours, respectively. This suggests a slow absorption and rapid metabolism of ASM 981.

The metabolite patterns in blood were highly complex with only a small contribution (about 5% of the AUC<sub>0-24 hr</sub> of total radioactivity) of the parent drug. The study report states that rabbits showed higher relative abundance of the numerous metabolites of medium polarity in the blood compared to rat, minipig and man. The metabolite patterns in the fetuses and blood were similar at 24 hours post dose. This indicates that the fetuses were exposed to both the parent drug and metabolites.

Urinary excretion of radioactivity in the 0-24 hour post dose interval was very low (0.6%). This indicates that the renal route of excretion after ASM 981 administration is marginal, similar to what has been observed in rat, minipig and man. The radioactivity in urine was composed exclusively of very polar material. No parent drug was found in the urine.

### Minipig ADME:

#### **Pharmacokinetic Study #8:**

*Absorption and disposition in minipig following single 5.7 μmol/kg (4.65 mg/kg) or 48.7 μmol/kg (39.6 mg/kg) oral (solid dispersion) and 2.3 μmol/kg (1.85 mg/kg) intravenous doses of [<sup>3</sup>H] SDZ ASM 981*

Study Title: Absorption and disposition in minipig following single 5.7 μmol/kg (4.65 mg/kg) or 48.7 μmol/kg (39.6 mg/kg) oral (solid dispersion) and 2.3 μmol/kg (1.85 mg/kg) intravenous doses of [<sup>3</sup>H] SDZ ASM 981

Study No: M-14/303-133  
Conducting laboratory: Sandoz Pharma LTD, Basel, Switzerland  
Study release date: August 26, 1996  
GLP compliance: No

The objective of this study was to determine the absorption and disposition characteristics of ASM 981 in the minipig. [<sup>3</sup>H]ASM 981 was administered to 3 male Gottingen minipigs/dose group as a single intravenous dose (1.85 mg/kg), a single low oral dose (4.65 mg/kg) and a single high oral dose (39.6 mg/kg). The low and high oral doses were selected to approximately correspond with the low and high dose (5 and 45 mg/kg, respectively) administered in a 2 week repeat dose toxicology study conducted in minipigs.

The absorption, disposition and mass balance were assessed in groups of 3 minipigs after each dosing regimen. Blood samples were collected at 0.08 (iv only), 0.15 (iv only), 0.5, 1, 2, 4, 6, 8, 12, 24, 31, 48, 72, 120, 168, 240, 336 and 456 hours (low oral dose only) post dose. Urine and feces samples were collected during the 0-7 hr (urine only), 7-24 hr (urine only), 0-24 hr (feces only) and then over consecutive 24 hour periods up to 264 hours. Cage washes were obtained for radioactivity level measurement. Blood concentrations of radioactivity and parent drug were determined by \_\_\_\_\_

Radioactivity in urine, feces and cage washes was measured by \_\_\_\_\_

Metabolite profiles were determined by HPLC \_\_\_\_\_

The study report states that ~1% of the dose was metabolized to tritiated water. Mean pharmacokinetic parameters (mean  $\pm$  SD) for total radioactivity and for the parent compound following a single intravenous dose of 1.85 mg/kg, a single low oral dose of 4.65 mg/kg or a single high oral dose of 39.6 mg/kg [<sup>3</sup>H] ASM 981 are provided in the following table.

Measure	C <sub>max</sub> (pmol/ml)			T <sub>max</sub> (hours)			AUC (pmol·hr/ml)		
	iv	Low po	High po	iv	Low po	High po	iv	Low po	High po
Radio-activity	--	266 $\pm$ 207	581 $\pm$ 198	--	23 $\pm$ 22	29 $\pm$ 37	15170 $\pm$ 1232	34727 $\pm$ 15966	86984 $\pm$ 12527
ASM 981	1619 $\pm$ 471	152 $\pm$ 224	338 $\pm$ 129	--	3.5 $\pm$ 2.8	3.0 $\pm$ 2.0	4002 $\pm$ 373	4827 $\pm$ 7122	4892 $\pm$ 2274

The total absorption following low and high oral doses of [<sup>3</sup>H] ASM 981 was 91  $\pm$  42% and 28  $\pm$  4%, respectively. The estimated bioavailability appeared to be higher after the low oral dose (48  $\pm$  71%) than after the high oral dose (7.5  $\pm$  3.5%). The parent drug (ASM 981) accounted for 22% of the total radioactive AUC after the iv dose, 14% after low oral dose and 6% after high oral dose. This result indicates that ASM 981 underwent extensive metabolism after administration.

Metabolite patterns were determined in blood after the intravenous and two oral doses. The radiochromatograms of pooled blood extracts showed essentially a \_\_\_\_\_ peak for ASM 981 (tautomers), a large number of minor metabolites eluting between 50 and 90 minutes, and a front peak (\_\_\_\_). ASM 981 represented the major drug related component at early times both after intravenous and oral administration. ASM 981 accounted for 73%, 37% and 15% of the

total AUC after intravenous, low oral and high oral dose administration, respectively, over the time interval collected for each dose. The peaks in the middle region of the chromatograms were too numerous to be completely separated and only — of the peaks — were abundant enough or sufficiently separated to be observed consistently. — was very minor and — was seen in slightly higher concentrations. — was more prominent in the animals of the high oral dose group.

The metabolites and parent drug areas under the curve in blood after intravenous (1.85 mg/kg), low oral (4.65 mg/kg) and high oral (39.6 mg/kg) dosing is provided in the following table.

Peak	Intravenous AUC <sub>(0-48 hr)</sub>		Low Oral AUC <sub>(0-168 hr)</sub>		High Oral AUC <sub>(0-240 hr)</sub>	
	nmol·hr/ml	%	nmol·hr/ml	%	nmol·hr/ml	%
—	0.55	10	2.7	23	16.2	35
—	0.07	1	nd	nd	0.2	1
—	0.32	6	0.3	3	2.8	6
ASM 981 <sup>a</sup>	3.89	73	4.3	37	6.9	15
Residual <sup>b</sup>	0.54	10	4.4	37	19.8	43

nd – not detected

a – sum of major and minor tautomer

b – minor metabolites not separated chromatographically

Less than 3% of the radioactivity administered was excreted into urine following intravenous or oral administration. The metabolite patterns were similar after intravenous and oral administration. The radiochromatogram showed a very broad hump around 50 minute retention time. Between 11 and 18% of this peak was due to tritiated water. The rest represented very polar metabolites. In addition, traces of ASM 981 (~0.02% of dose) and — minor metabolite peaks were detected on top of the broad hump after high oral dose but not after the low oral or intravenous doses. The metabolite patterns in feces extracts consisted mainly of a very broad hump between 20 and 80 minutes retention time. This peak probably represents numerous unresolved metabolites. Parent drug was detected in feces after oral administration, especially after high oral dose, but not following intravenous dosing. Total percent of radiochromatogram contributed to ASM 981 was 0%, 2% and 10% after iv, low oral and high oral dose administration, respectively. The ASM 981 detected in feces was due to unabsorbed ASM 981.

Drug related material was excreted slowly and almost exclusively in the feces independent of the route of administration. The mean fecal and urinary excretion was  $87.8 \pm 2.7$  and  $2.4 \pm 0.4\%$  of the dose, respectively, following iv administration. A mean of  $87.2 \pm 0.8$  and  $1.6 \pm 0.4\%$  of the dose was excreted in feces and urine, respectively, after low oral dose administration. A mean of  $82.2 \pm 25.0$  and  $1.2 \pm 0.2\%$  of the dose was excreted in feces and urine, respectively, after high oral dose administration. The recovery of radioactivity was  $91.6 \pm 2.4$ ,  $90.1 \pm 1.3$  and  $91.3 \pm 14.4$  in the iv, low oral and high oral dose studies, respectively.

#### Pharmacokinetic Study #9:

*Absorption and disposition in minipigs after a single topical (dermal) application [<sup>3</sup>H]- labeled SDZ ASM 981 formulated as 1% FMF cream*

Study Title: Absorption and disposition in minipigs after a single topical (dermal) application [<sup>3</sup>H]- labeled SDZ ASM 981 formulated as 1% FMF cream  
Study No: M-15/303-195  
Conducting laboratory: Novartis Pharma AG, Basel, Switzerland  
Study release date: April 29, 1998  
GLP compliance: No

The objective of this study was to determine the absorption through the skin and disposition characteristics of ASM 981 in minipigs. [<sup>3</sup>H]ASM 981 was administered to two male Gottingen minipig as a single topical dose (20 mg/kg/day of 1% ASM 981 FMF, 2 grams applied to 20% of total body surface). The treatment duration was for 22 hours. The application site was protected with a porous gauze dressing and retained by a netlike body stocking attached to a neck collar.

Blood samples were collected prior to treatment (-22 hr), at 8 hours after application of the cream (-14 hr), at the end of the 22 hr application period (0 hr) and at 1, 2, 4, 6, 8, 11, 24, 48, 120, 168 and 240 hours after washing of the application site. A portion of the blood sample was measured for total radioactivity and a portion was analyzed for metabolite profile. Skin biopsies (8 mm in diameter, ~3.5 mm thick) were obtained after stripping of the stratum corneum at the site of application at 0, 24, 48, 120 and 240 hours post washing and at sites remote from the application site at 0, 24 and 120 hours post washing. Skin biopsies were analyzed for either total radioactivity or for metabolite profiles. Stratum corneum strips were analyzed for total radioactivity.

Urine, feces and cage wash water were collected separately and quantitatively during the 24 hours pretreatment period (urine and feces only), during the 22 hour treatment period and then for 24 hour consecutive periods up to 240 hour post end of application. The gauze dressings and body stockings were obtained at the end of the 22 hour treatment period. The cleaning materials were collected after washing the skin and the skin at the application site was dissected out after sacrifice of the animals (240 hours post end of application).

Radioactivity levels in blood, urine, feces, whole skin at the application site, skin biopsies, skin strips, extracts of gauze dressings, body stockings and cleaning materials and cage wash water were measured by \_\_\_\_\_ . Metabolite profiles from blood, urine and feces were determined by HPLC \_\_\_\_\_ ASM 981 levels in blood were determined by the \_\_\_\_\_

In total, 91.6-94.9% of the radioactive dose was recovered in the gauze dressings, body stockings and cleaning materials at the end of the 22 hour exposure period. The major fraction was found in the gauze dressings and body stockings (85.7-88.4%). All blood levels of radioactivity were below the limit of detection (— pmol/g). However, due to the lower quantitation limit of the — method (~ pmol/ml), ASM 981 was detected in blood up to 11-