

24 hours post application. The mean C_{\max} of 0.44 ± 0.54 pmol/ml was observed at the mean T_{\max} of 4 hours. The $AUC_{0-24 \text{ hr}}$ for ASM 981 was equal to 9.31 ± 11.49 pmol·hr/ml. The bioavailability was estimated to be ~0.03%.

The amount of radioactivity in the epidermis and dermis at the application site remained essentially constant in the time interval 0-240 hours post end of application. The radioactivity that remained in the skin represented mostly ASM 981. This suggests that a drug depot of ASM 981 may remain in the skin after application. Much higher concentrations of radioactivity (~20X; mainly ASM 981) were present in the epidermis compared to the dermis. The level of radioactivity in the stratum corneum decreased slowly over the 240 hour measurement period. The levels of radioactivity in the skin remote from the application site was ~2% of that noted at the application site.

The metabolite patterns in the blood extracts were complex. The metabolites appeared to be of a polar nature and seemed to have been similar to ones that had been noted after oral dosing. However, definitive analysis was not possible due to the low levels of metabolites in the blood samples. The study report simply states that the metabolite pattern in feces was highly complex. Overall mass balance for this experiment was essentially complete (94.3-95.9% of the dose).

In Vitro Blood/Plasma Partitioning and Protein Binding Studies:

Pharmacokinetic Study #10:

In vitro distribution and stability of [³H] SDZ ASM 981 in rat, mini-pig and human blood

<u>Study Title:</u>	<i>In vitro distribution and stability of [³H] SDZ ASM 981 in rat, mini-pig and human blood</i>
<u>Study No:</u>	M-16/303-039
<u>Conducting laboratory:</u>	Sandoz Pharma LTD, Basel, Switzerland
<u>Study release date:</u>	March 23, 1995
<u>GLP compliance:</u>	No

The objective of this study was to assess the distribution of [³H] ASM 981 between blood cells and plasma. In addition, the stability of [³H] ASM 981 in fresh rat, minipig and human blood *in vitro* was determined in this study. This data would be used to determine whether the absorption and disposition parameters for [³H] ASM 981 *in vivo* in these species would be best determined from blood or plasma samples.

The percentage of [³H] ASM 981 in plasma was concentration dependent. The percentage increased from 56 to 82%, 8 to 46% and 12 to 71% in rat, minipig and human blood, respectively, over the concentration range tested in this study (1 – 1000 ng/ml). Therefore it was determined that blood would be more suitable than plasma for the *in vivo* determination of the absorption and disposition parameters of ASM 981. [³H] ASM 981 was stable in rat, minipig and human blood over the concentration range tested in this study (1 – 1000 ng/ml).

Pharmacokinetic Study #11:

In vitro protein binding of [³H]ASM981 in rats, minipigs and humans

Study Title: *In vitro* protein binding of [³H]ASM981 in rats, minipigs and humans
Study No: M-18/303-211
Conducting laboratory: _____
Study release date: September 16, 1998
GLP compliance: No

The extent of *in vitro* protein binding of ASM 981 in plasma proteins of rats, minipigs and humans was investigated by _____ using ³H-labeled compound.

The extent of plasma protein binding of [³H] ASM 981 was concentration independent over the concentration range 1 – 1000 ng/ml in rats, minipigs and humans. The fraction bound in humans and minipigs was similar (81% and 83%, respectively). A slightly higher fraction bound was noted in rats (90%).

Pharmacokinetic Study #12:

ASM 981: In vitro blood distribution and protein binding of ³H-labeled ASM981 in mouse and rabbit and stability in mouse and rabbit blood

Study Title: ASM 981: *In vitro* blood distribution and protein binding of ³H-labeled ASM981 in mouse and rabbit and stability in mouse and rabbit blood
Study No: M-17/R00-1510
Conducting laboratory: Novartis Pharma AG, Basel, Switzerland
Study release date: September 8, 2000
GLP compliance: No

The first objective of this study was to investigate the *in vitro* blood distribution and the plasma protein binding of ASM 981 in mouse and rabbit. The second objective of this study was to investigate the *in vitro* stability of ASM 981 in mouse and rabbit blood.

The distribution of [³H] ASM 981 between blood cells and plasma was essentially concentration independent in mouse over the concentration range of 1 – 1000 ng/ml. The test compound was virtually completely located in the plasma compartment. The fraction of [³H] ASM 981 in mouse plasma was 96 ± 2%. In contrast, the blood distribution of [³H] ASM 981 showed a pronounced concentration dependency in rabbits. The plasma fraction increased from 29% at 1 ng/ml to 79% at 1000 ng/ml. The rabbit behaves similarly to rats, minipigs and humans that demonstrated a concentration dependent blood distribution as well.

Plasma protein binding of [³H] ASM 981 was concentration independent in mice and rabbits over the concentration range of 3 – 1000 ng/ml. The test compound was moderately bound to plasma proteins in mice (88%) and rabbits (83%). The plasma protein binding results noted in rats, minipigs and humans were similar.

demethylation near position 23 or 25. This demethylation accounted for least 5 metabolites noted in the radiochromatogram due to slowly interconverting tautomeric structures. Additional minor metabolites, partially co-eluting with the demethylated metabolites, were found to be products of oxygenations and a dehydration. A few products of multiple reactions were found that had undergone either two demethylations or a demethylation and a dehydration. With increased incubation time, it can be anticipated that products of multiple reactions will become more abundant and numerous, resulting in the highly complex metabolite patterns noted in human liver microsomal incubations at longer time periods.

ASM 981 appears to be metabolized by liver subcellular fractions to essentially the same biotransformation products as found *in vivo* in blood and feces. Metabolite patterns generated *in vitro* under conditions of low turnover seem to be similar to those in blood. With increasing turnover, a gradual shift to more polar metabolites and to more complex profiles is observed which more closely resembles profiles noted in feces samples.

Pharmacokinetic Study #14:

Metabolism by rat and human liver microsomes and by human liver S12 fractions, Amendment No. 1

Study Title: Metabolism by rat and human liver microsomes and by human liver S12 fractions, Amendment No. 1
Study No: M-20/R97-541-01
Conducting laboratory: Novartis Pharma AG, Basel, Switzerland
Study release date: August 14, 2000
GLP compliance: No

The purpose of this amendment was to clarify the identification of two metabolites noted in the previous study. These two metabolites were noted as being formed after dehydration in the original report. However, the amendment states that dehydrations of ASM 981 by human liver fractions seemed unlikely. Instead, these metabolites are attributed to a hydrolytic dechlorination (or an oxidative dechlorination combined with a ketone reduction), which would result in the same change in molecular weight as a dehydration (i.e., a loss of 18 mass units).

Pharmacokinetic Study #15:

CYP3A4/5 is the main cytochrome P450 isozyme involved in the microsomal biotransformation of ASM 981

Study Title: CYP3A4/5 is the main cytochrome P450 isozyme involved in the microsomal biotransformation of ASM 981
Study No: M-21/R98-1075
Conducting laboratory: Novartis Pharma AG, Basel, Switzerland
Study release date: April 8, 1999
GLP compliance: No

The objective of this study was to determine which human cytochrome P450 enzyme metabolize ASM 981 at therapeutically relevant concentrations. In addition, the potential of selected comedications to inhibit the human microsomal metabolism of ASM 981 was evaluated.

The P450 dependant metabolism of [³H] ASM 981 was investigated *in vitro* using human liver microsomes. The identity of the cytochrome P450 isozyme(s) involved was assessed by the use of selected recombinant human microsomes and specific cytochrome P450 inhibitors. Twelve potential comedications of ASM 981 were tested for their potential inhibitory drug interaction within a concentration range of 0-100 μM.

The biotransformation of [³H] ASM 981 was evaluated by the measure of [³H] ASM 981 disappearance in comparison with control incubations. Human liver microsomes catalyzed predominantly oxidative O-demethylations of [³H] ASM 981. The reaction rate was dependent on the [³H] ASM 981 concentration in the range 0.1 to 8 μM and followed an apparent single enzyme Michalis-Menten kinetic with K_m and V_{max} values of 0.7 μM and 394 pmoles [³H] ASM 981 metabolized/min/mg protein, respectively.

In a panel of ten individually expressed P450 isozymes, only CYP3A4/5 catalyzed efficiently the biotransformation of [³H] ASM 981. Specific inhibition of the reaction with ketoconazole or a monoclonal antibody inhibitory to CYP3A4/5 confirmed the predominant role of CYP3A4/5 in the human microsomal biotransformation of [³H] ASM 981.

Cimetidine, theophylline, fluoxetine or salbutamol (100 μM each) did not effect the microsomal biotransformation of 1 μM [³H] ASM 981. Erythromycin, prednisolone and warfarin were weak inhibitors (IC₅₀ > 100 μM). Ketoconazole, budesonide, terfenadine, ethynylestradiol and astemizole inhibited the reaction IC₅₀ values of 0.25, 14, 24, 52 and 54 μM, respectively. The results from this experiment suggest that CYP3A4/5 plays a major role in the metabolism of [³H] ASM 981 in humans.

Pharmacokinetic Study #16:

Species comparison of hepatic metabolism in vitro

<u>Study Title:</u>	Species comparison of hepatic metabolism <i>in vitro</i>
<u>Study No:</u>	M-22/R98-192
<u>Conducting laboratory:</u>	Novartis Pharma AG, Basel, Switzerland
<u>Study release date:</u>	July 27, 2000
<u>GLP compliance:</u>	No

The objective of this study is to compare the hepatic biotransformation of ASM 981 in CD-1 mouse, Wistar rat, New Zealand White rabbit, Yuctan minipig and human. Potential metabolites can be obtained in higher quantities and greater purity from *in vitro* studies. This allows for improved conditions for the characterization of the metabolites by —

[³H] ASM 981 was incubated with liver microsomes from humans and from all four test species used in the toxicity studies (mouse, rat, rabbit and minipigs). The metabolites were characterized by —

The metabolism of ASM 981 in human microsomes illustrated that the main primary biotransformation of ASM 981 was O-demethylation near position 23 or 25 of the macrocycle (designated Dx_1). The — data did not allow to distinguish between the two possibilities. Additional metabolic reactions, proposed on the basis of the — data, included:

- O-demethylations at the additional two methoxy groups
- Hydroxylation, predominantly at one of the carbon bound methyl groups
- Oxidative dechlorination (minor pathway)
- Hydrolytic dechlorination or oxidative dechlorination followed by ketone reduction (very minor pathway)
- Oxidation of a methylene to a keto group (very minor pathway)

Second and higher generation metabolites resulted mainly from combinations of the observed primary biotransformation reactions. In general, O-demethylations clearly predominated over the other metabolic pathways.

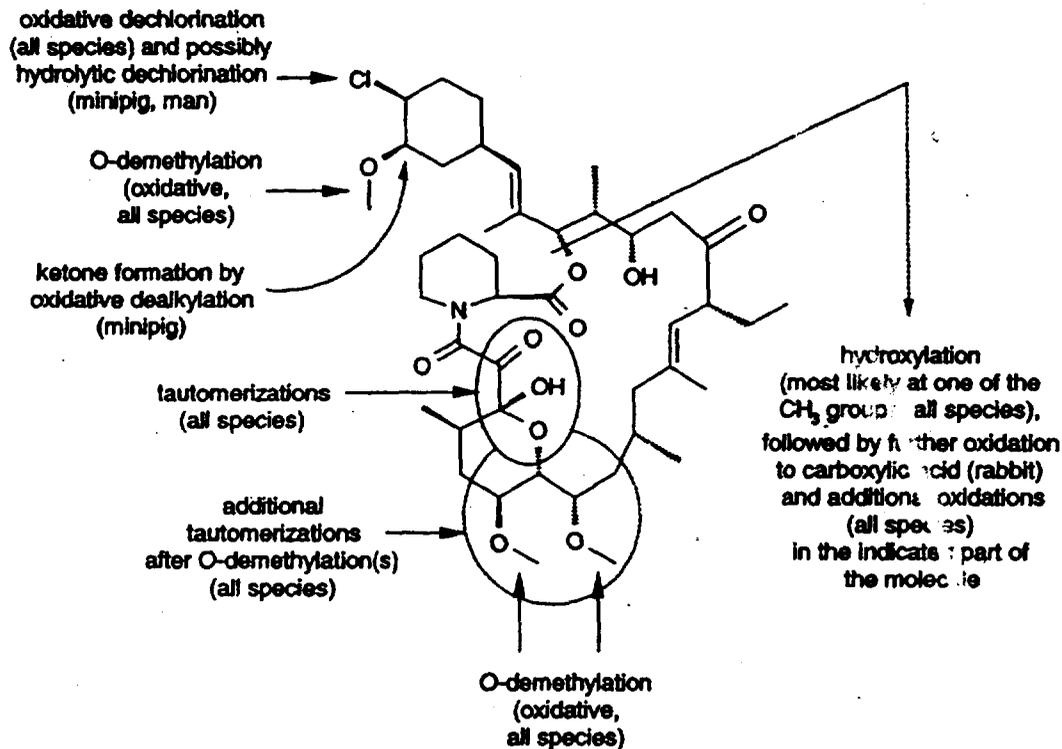
The animal liver microsomes generated essentially the same metabolites as the human ones, though at different relative rates. As the only major exception, the rabbit liver microsomes oxidized ASM 981 to a major and minor carboxylic acid metabolite which were absent in the other species (or present in trace amounts only). The similarities between humans and animals in the metabolism of ASM 981 were as follows:

- All primary metabolites formed by the human liver microsomes were formed also by the liver microsomes from the four animal species investigated
- The major primary metabolite in man (Dx_1) was also among the most prominent primary metabolites formed by three of the four animal species (except the rabbit)
- Essentially all second and higher generation metabolites in man were formed by at least one of the animal species

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The complexity of the metabolism of ASM 981 in animals and man is due to the occurrence of numerous parallel and consecutive biotransformations and the existence of multiple tautomeric forms of the metabolites. The various possible sites of biotransformation of ASM 981 in humans and the various animals species is depicted in the following figure (scanned from the NDA submission).

Sites of biotransformation of ASM981 by mouse, rat, rabbit, minipig and human liver microsomes and tautomerizations:



Pharmacokinetic Study #17:

Evaluation of ASM981 as an inhibitor of human P450 enzymes

Study Title: Evaluation of ASM981 as an inhibitor of human P450 enzymes
Study No: M-23/R97-532
Conducting laboratory: Novartis Pharma AG, Basel, Switzerland
Study release date: August 5, 1998
GLP compliance: No

The objective of this study was to evaluate the ability of ASM 981 to inhibit the major P450 enzymes in human liver microsomes. The data generated from this study would determine the potential for ASM 981 to inhibit the metabolism of other drugs. To evaluate ASM 981 as a direct acting (metabolism independent) reversible inhibitor of P450 activity, human liver microsomes were incubated with marker substrates at a concentration equal to K_m in the

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presence or absence of ASM 981 at concentrations ranging from 0.1 to 10 μM . In addition, ASM 981 was evaluated for its ability to function as a metabolism dependent irreversible inhibitor. To assess this possibility, ASM 981 was pre-incubated with human liver microsomes and NADPH for 15 minutes to allow for the generation of metabolites that could inhibit cytochrome P450 non-competitively. Known reversible or metabolism dependent inhibitors of P450 activity were included as positive controls when possible. The major P450 enzymes evaluated in this study along with the corresponding substrate are listed below.

- CYP1A2 7-Ethoxyresorufin O-dealkylation
- CYP2A6 Coumarin 7-hydroxylation
- CYP2B6 7-Ethoxy-4-trifluoromethylcoumarin O-dealkylation
- CYP2C9 Diclofenac 4'-hydroxylation
- CYP2C19 S-Mephenytoin 4'-hydroxylation
- CYP2D6 Dextromethorphan O-demethylation
- CYP2E1 Chlorzoxazone 6-hydroxylation
- CYP3A4/5 Testosterone 6 β -hydroxylation
- CYP4A9/11 Lauric acid 12-hydroxylation

The following conclusions could be drawn from the results of this experiment concerning the ability of ASM 981 to act as a CYP450 inhibitor.

- 1) ASM 981 has little or no capacity to inhibit CYP1A2, CYP2A6, CYP2B6, CYP2E1 or CYP4A9/11
- 2) ASM 981 appears to be a competitive inhibitor of CYP3A4/5 with a $K_i = 1.0 \mu\text{M}$
- 3) ASM 981 appears to be a non competitive inhibitor of CYP2C9 with a $K_i = 22 \mu\text{M}$
- 4) ASM 981 appears to be an inhibitor of CYP2C19 and CYP2D6 with estimated IC_{50} values of 13 and 15 μM , respectively.
- 5) ASM 981 had little or no capacity to function as an irreversible metabolism dependent inhibitor of any of the P450 enzymes examined (with the possible exception of CYP3A4/5)

The following assumptions were drawn from the results for this experiment. ASM 981 is unlikely to reduce the *in vivo* clearance of drugs mainly cleared through metabolism by CYP1A2, CYP2A6, CYP2B6, CYP2E1 or CYP4A9/11. ASM 981 may possibly reduce the clearance of coadministered drugs that are mainly metabolized by CYP3A4/5, CYP3C9, CYP2C19 and/or CYP2D6.

Pharmacokinetic Study #18:

Tautomer interconversion of ASM981 and metabolites

Study Title: Tautomer interconversion of ASM981 and metabolites
Study No: M-24/R99-2683
Conducting laboratory: Novartis Pharma AG, Basel, Switzerland
Study release date: August 4, 2000
GLP compliance: No

The objective of this study was to investigate the rate of tautomer interconversion for the parent drug and a major human metabolite (Dx_1). The three tautomers of [³H] ASM 981 were isolated by HPLC. The major human metabolite (Dx_1) was isolated by HPLC from pooled incubated of [³H] ASM 981 with human liver S12 fractions. The isolated components (tautomers of ASM 981 and metabolite Dx_1) were kept for different periods of time at room temperature or at elevated temperature in the solvent in which they were collected off the HPLC. After the various time points, an aliquot was re-injected onto the HPLC column for analysis.

The three tautomers of ASM 981 were found to gradually interconvert, finally resulting in one and the same equilibrium mixture, independent of the tautomer used at the start of the experiment. The structures of the tautomers were determined as a 6-ring hemiacetal (main tautomer ASM_3), a 7-ring hemiacetal (ASM_2) and a ring-opened tricarbonyl component (ASM_1).

The O-demethylated metabolite Dx_1 was shown to be in tautomeric equilibrium with the known metabolites Dx_2 and Dx_3. This implied that all three were O-demethylated at the same position (O-demethylation near position 23 or 25 of the macrocycle).

The results for this experiment support the hypothesis that the complexity of the metabolite patterns of ASM 981 in animals and man is partially due to multiple tautomeric structures of the same metabolite. The rate of tautomer interconversion was found to be slow enough to allow the observation of the tautomers as separate chromatographic peaks.

Pharmacokinetic Study #19:

Metabolism of ASM981 in human skin in vitro

<u>Study Title:</u>	Metabolism of ASM981 in human skin <i>in vitro</i>
<u>Study No:</u>	M-25/R00-1030
<u>Conducting laboratory:</u>	Novartis Pharma AG, Basel, Switzerland
<u>Study release date:</u>	July 28, 2000
<u>GLP compliance:</u>	No

The objective of this study was to measure the extent of metabolism of ASM 981 *in vitro* using fresh human skin. [³H] ASM 981 was applied as the 1% final market form of the cream (~10 mg cream/cm²) to the epidermal surface (stratum corneum) of fresh human skin. The incubations were performed at 37°C and the epidermal side was exposed to a gas atmosphere (95% O₂, 5% CO₂) while the opposite (dermal) side of the skin was in contact with a culture medium that was agitated by a rolling movement. After different incubation times (up to 24 hours), the cream remaining on the surface of the skin was removed and the media, skin tissues and washing fluids were collected for measuring radioactivity. Selected samples were analyzed by HPLC

The initial penetration of ASM 981 into the skin was rapid, but saturation of the skin appeared to have been reached after 0.5 hour, even though >90% of the applied ASM 981

remained unabsorbed in the surface of the skin up to 24 hours. The radiolabeled material in the skin (both epidermis and dermis) at 24 hours represented exclusively unchanged [³H] ASM 981. The concentration in the epidermis at 24 hours (62 nmol/g) was ~6X higher than the amount in the dermis (10 nmol/g).

Permeation of ASM 981 through the skin into the culture medium did not occur or was extremely low. The radiolabeled material detected in the medium at 24 hours (_____ of the applied radioactivity, _____ of the radioactivity in the skin) consisted almost exclusively of highly polar components. The report states that they seemed to represent radiolabeled impurities (eg, _____ or their metabolites, not metabolites of [³H] ASM 981. [³H] ASM 981 was hardly detectable in the medium.

These results suggest that no metabolism of ASM 981 occurred in human skin under the conditions of this study.

Pharmacokinetic Study #20:

Pharmacological activity of human metabolite pools

Study Title: Pharmacological activity of human metabolite pools
Study No: M-26/R99-2221
Conducting laboratory: Novartis Pharma AG, Basel, Switzerland
Study release date: September 15, 2000
GLP compliance: No

The objective of this study was to determine whether the metabolites of ASM 981 might contribute to the pharmacological activity of the compound after oral administration. Metabolites were generated by incubating [³H] ASM 981 with human liver microsomes. Three incubation times were selected (5, 15 and 60 minutes) to cover the full range of metabolites. The metabolite patterns (radiochromatograms) showed a continuous increase in the number and in the average polarity of the metabolites with increasing incubation time. The study report states that the metabolite profile after 5 minutes closely resembled that found *in vivo* in human blood between 1 and 8 hours after oral administration. The study report states that the 60 minute incubate contained a complex mixture of metabolites similar to that found in human feces (main route of excretion). In addition, the study report states that highly polar metabolites formed gradually *in vitro* were probably identical with those excreted in human urine (2-3% of dose only). Therefore, the metabolites formed *in vitro* can be assumed to be the same as those occurring *in vivo* after oral administration. Because of the complexity of the metabolism and the absence of clearly dominating biodegradation products, no individual metabolites but only pools were tested pharmacologically. The metabolites in each of the three incubates were fractionated into two pools. One pool contained highly polar metabolites (Fraction 1) and one containing the moderately and non-polar metabolites (Fraction 2). Pharmacological (immunosuppressive) activity was measured using the _____

Fraction 1 was devoid of immunosuppressive activity in the _____
Fraction 2 showed moderate to low immunosuppressive activity. The immunosuppressive

activity of fraction 2 decreased with increased incubation time (i.e., with increasing polarity of the metabolites from non-polar to moderately polar). The average immunosuppressive activity of the metabolites in fraction 2 was 9%, 4% and 0.5% of the activity of ASM 981 in the 5, 15 and 60 minute incubates, respectively.

Based on the results of this study it appears that moderately to highly polar metabolites of ASM 981 are pharmacologically inactive. In contrast, some of the non-polar metabolites of ASM 981 appear to have some pharmacological (immunosuppressive) activity. However, due to the low concentration of the metabolites of ASM 981 in the blood relative to the parent drug, it appears unlikely that the metabolites of ASM 981 provide a major contribution to the pharmacological activity of ASM 981 after oral administration.

Pharmacokinetic Study #21:

Mechanistic transport studies across Caco-2 cell monolayers

<u>Study Title:</u>	Mechanistic transport studies across Caco-2 cell monolayers
<u>Study No:</u>	M-27/R00-1674
<u>Conducting laboratory:</u>	Novartis Pharma AG, Basel, Switzerland
<u>Study release date:</u>	August 10, 2000
<u>GLP compliance:</u>	No

The objective of this study was to investigate the involvement of an active transporter in the movement of ASM 981 across the intestinal barrier. Another objective of this study was to determine if a particular transporter may be involved with the movement of ASM 981 across the intestinal barrier. The human intestinal cell line Caco-2 was grown on a permeable filter support. The concentration dependent compound transport (0.01, 10 and 50 μM ASM 981) across Caco-2 cell monolayers was measured from the apical to basolateral side and from the basolateral to apical side. In addition, apical to basolateral transport experiments with 0.1 μM ASM 981 were conducted in the presence of potent efflux system inhibitors CsA (10 μM) and Verapamil (100 μM).

No apical to basolateral transport was detectable for the low concentration of ASM 981 (0.01 μM) over a 120 minute time period. Significant basolateral to apical transport occurred with a permeability value of about 24×10^{-5} cm/min for the low concentration of ASM 981 (0.01 μM). A significant increase in apical to basolateral transport was observed between 10 and 50 μM ASM 981. The permeability values in both transport directions appeared to converge at 10 μM ASM 981 (permeability value = 4×10^{-5} cm/min). Addition of Verapamil or CsA resulted in a significant increase of apical to basolateral transport of the low concentration of ASM 981 (0.01 μM) that approached the bidirectional permeability levels determined for the mid and high concentrations of ASM 981 (10 and 50 μM). The results of this experiment suggest that ASM 981 is a moderately permeable compound and may be a substrate for the prominent efflux Pgp system.

Pharmacokinetic summary:

The nonclinical pharmacokinetic data obtained from studies conducted with ASM 981 are summarized in the five sections below titled: a) pharmacokinetic parameters, b) absorption, c) distribution, d) metabolism and e) excretion.

Pharmacokinetic parameters:

The pharmacokinetic parameters of ASM 981 and metabolites in blood after intravenous, oral or dermal dosing with [³H] ASM 981 are summarized in the following table.

Species	Dose (mg/kg)	Route and frequency	C _{max} (ng/ml)	AUC _{0-infinity} (ng·hr/ml)
Mouse	15	po, single	199	960
	3	iv, single	--	2753
Rat	1	iv, single	--	303
	5	po, single	148	415
	100	po, single	1678	22487
	3	dm, 5 days	<LOQ	<LOQ
Minipig	1.85	po, single	--	3244
	4.65	po, single	123	3912
	39.6	iv, single	274	4775

Absorption:

Oral absorption of ASM 981 was highly variable in all species and appeared to be dose dependent. Single oral doses of [³H] ASM 981 were administered to rats, mice and minipigs. Absorption of [³H] ASM 981 was moderate in mice (37% of a 15 mg/kg dose), and moderate to high in rats (55% of a 5 mg/kg dose and 93% of a 100 mg/kg dose) and minipigs (91% of a 39.6 mg/kg dose and 28% of a 4.65 mg/kg dose). The rate of onset of oral absorption of 20 mg/kg [³H] ASM 981 dose in pregnant rabbits appeared to be much slower than the other species tested.

Dermal penetration of pimecrolimus was very low in rats and minipigs. When [³H] ASM 981 cream formulations (0.3% formulation B on rats for 5 days or single dose 1.0 % FMF on minipig) were applied to about 20% of the body surface area, only about 0.2 – 1.4% of the dose penetrated the skin (95% of which was in the epidermis). Blood levels were very low (less than 0.1 ng/ml). The overall bioavailability of ASM 981 after dermal administration was ~0.03%.

Distribution:

ASM 981 was stable at drug concentrations between 1 and 1200 ng/ml at 37°C for at least 1.5 hours in the blood of mouse, rat, rabbit, minipig and human. In the blood of species tested, with the exception of the mouse, the distribution of [³H] ASM 981 between blood cells and plasma was concentration dependent in the range of 1-1200 ng/ml. This suggests that there may be saturable high affinity/low capacity binding sites for ASM 981 in or on the blood cell. The proportion of radioactivity in the plasma ranged from 56 – 82% in the rat, 29-79% in the rabbit,

8-46% in the minipig and 12-71% in human. The proportion of radioactivity in the plasma was concentration independent in the mouse (96% over the concentration range tested).

After systemic administration (intravenous or oral) of [³H] ASM 981 to the rat, the highest levels of radioactivity were found in highly perfused organs (liver, heart, blood vessels and lung) and in lipid rich tissues (spleen, thyroid and adrenal glands). These organs may prove to be potential target organs of toxicity. After dermal administration of [³H] ASM 981 to mice, rats or minipigs, the major part of the applied dose remained in the skin. Following oral treatment of [³H] ASM 981 to pregnant rats (45 mg/kg, gestation days 13 and 17) or rabbits (20 mg/kg, gestation day 17), the levels of radioactivity in the fetus were approximately the same as that in the maternal blood indicating significant placental passage of ASM 981.

Metabolism:

The metabolism of ASM 981 was investigated in the mouse, rat, rabbit, minipig and human using [³H] ASM 981. ASM 981 was not metabolized in fresh human skin *in vitro*. Only unchanged drug was detected in the skin of minipigs after dermal administration of [³H] ASM 981 for up to 5 days post dose. These results suggest that skin does not contribute to the metabolic profile for ASM 981.

The parent compound was noted in blood as the main circulating component after oral or intravenous administration of [³H] ASM 981. In addition to the parent compound, many minor metabolites were noted in the blood. ASM 981 was eliminated from the body almost exclusively by metabolism. Essentially no parent drug was detected in feces after intravenous dosing and in urine after oral and intravenous dosing. Unchanged ASM 981 noted in the feces after oral dosing was attributed to unabsorbed compound. Feces, which represented the predominant excretory route, contained a large number of metabolites that could not be separated by HPLC. The metabolite patterns in feces were complex. Low concentrations of metabolites were present in the feces, which allowed for only a partial characterization of the metabolites by ———. Even though definitive metabolite structures could not be elucidated for all of the ASM 981 metabolites, a "fingerprint" metabolite pattern could be established which allowed correlation of metabolites across species.

Metabolism *in vivo* was similar across all species and closely resembled metabolism in liver fractions *in vitro*. Metabolism involved a multitude of parallel and consecutive pathways. In addition, multiple slowly interconverting tautomers of the metabolites were observed. The predominant metabolic pathways in man were O-demethylations. The main pathway in human liver microsomes was O-demethylation at position 23 or 25, forming the metabolite designated as Dx_1 ———. The two regioisomers could not be distinguished by ———. Second and higher generation metabolites resulted mainly from combinations of the observed primary biotransformation reactions.

Metabolite patterns formed by liver microsomes from four toxicity species and humans were essentially identical. Some differences in rates of metabolism were noted among the species. Essentially all second and higher generation metabolites in humans were formed by at least one of the animal species *in vitro*. This result supports that the selection of the species for toxicity testing were appropriate for ASM 981.

Limited pharmacological (immunosuppressive) activity was found with pooled ASM 981 metabolites formed by human liver microsomes using _____ Due to low blood concentrations, relative to parent drug, metabolites are unlikely to contribute to the overall pharmacological activity of the drug after oral dosing.

ASM 981 inhibited the metabolism of marker probe substrates that are mainly metabolized by CYP3A4/5 in human liver microsomes *in vitro*. ASM 981 had little or no effect on other cytochrome P450 enzymes. Therefore, the clearance of ASM 981 may potentially be affected by comedications that are known to be strong CYP3A4/5 inhibitors (e.g., ketoconazole like antifungal azoles). This would probably be a significant factor for oral administration of ASM 981 but not for dermal administration of ASM 981 due to limited systemic bioavailability after topical administration.

Excretion/elimination:

The decline of unchanged ASM 981 in the blood was multi-phasic in all species investigated following single intravenous and oral dosing of radiolabeled ASM 981. Short initial half-life ($t_{1/2}$) values ranging between 0.5 – 0.8 hours was noted in all species. This may have been attributed to distribution of ASM 981 into organs and tissues. This initial phase was followed by a more prolonged elimination phase with $t_{1/2}$ values of 4 hours in mice, 9.9 hours in rats and 7.9 hours in minipigs.

The elimination of ASM 981 from the body occurred almost entirely via metabolism. Independent of the route of administration, the major fraction of the absorbed dose was excreted slowly mainly via bile into the feces in all investigated species. Renal excretion generally accounted for less than 3% of the dose. The mass balance was almost complete in all species investigated with [^3H] ASM 981.

Pharmacokinetic conclusions:

The pharmacokinetics and metabolism of ASM 981 were investigated in the mouse, rat, rabbit and minipigs after topical, intravenous and oral administration. Systemic exposure to ASM 981 after topical exposure was much lower than after oral administration. The metabolism of ASM 981 *in vitro* and *in vivo* in animals and humans was very complex and qualitatively similar in all investigated species. The metabolism of ASM 981 was mainly catalyzed by the CYP3A enzyme subfamily. The main pathway in human liver microsomes was O-demethylation at position 23 or 25, forming the metabolite designated as Dx_1 _____. The metabolites formed showed low activity in an *in vitro* pharmacological assay and were considered not to contribute significantly to the overall pharmacological activity of ASM 981 *in vivo*. ASM 981 was eliminated almost exclusively by metabolism with subsequent biliary excretion into feces. Unchanged ASM 981 represented the major drug related compound in blood and was accompanied by numerous very minor metabolites. ASM 981 was not metabolized or degraded during skin permeation.

TOXICOLOGY:

Acute Toxicology summary:

The acute toxicity of ASM 981 was assessed by the oral and iv routes in mice and rats and by dermal administration in rats. The results of the acute toxicology studies are summarized in the following table.

Species, Strain	Route	Dose (mg/kg)	LD ₅₀ (mg/kg)
Mouse, CD-1	Oral/Gavage	600	> 600
Mouse, CD-1	iv	50,100,130	>130
Rat, Wistar	Dermal	2000	> 2000
Rat, Wistar	Oral/Gavage	600	> 600
Rat, Wistar	iv	50, 75, 100	68.1

ASM 981 showed low acute toxicity potential. No lethality was observed after single oral doses of 600 mg/kg in either mice or rats. Higher doses than 600 mg/kg were not administered due to limited solubility of ASM 981 lyophilisate in the vehicle. Intravenous administration to mice caused muscle spasms and death at doses of ≥ 100 mg/kg. Intravenous administration to rats caused sedation, dyspnea and lethality at ≥ 50 mg/kg. The cause of death was not determined in these studies. Single doses of 2000 mg/kg ASM 981 applied for 24 hours to skin of rats under a semi-occlusive dressing elicited no lethality or toxic signs. The vehicle for ASM 981 in the acute dermal rat study was not the FMF cream.

Repeat Dose Toxicology Studies:**Oral Mouse:****Repeat Dose Toxicology Study #1:**

Tissue concentration following a 2 week oral (solid dispersion) and dermal treatment (ethanol solution) in mice

Study Title: Tissue concentration following a 2 week oral and dermal treatment in mice
Study No: T-18/203-180
Contract Study No: SAZ 592/970986
Volume #, and page #: 17, 5-1117
Conducting laboratory: _____
Date of study initiation: 1/28/97
GLP compliance: Yes
QA- Report: Yes (X) No ()
Drug, and lot#: ASM 981 – batch# 96913

Formulation/vehicle: Oral treatment – Aqueous solution containing 0.5% Poloxamer 188 and 3.5% hydroxypropylmethyl cellulose
Dermal Treatment – Ethanol

Methods:

Test article was administered orally (via gavage) or dermally on a daily basis for 14 days. The dermal dose was applied as a thin uniform layer to the clipped dorsum of the mouse.

Dosing:

- *species/strain:* CD-1 mice
- *#/sex/group or time point:* 25 males/group
- *satellite groups used for toxicokinetics or recovery:* N/A
- *age:* 7 weeks
- *weight:* 20 – 36 grams
- *doses in administered units:* 50 mg/kg ASM 981 (oral and dermal)
- *route, form, volume, and infusion rate:* oral, liquid suspension, 10 ml/kg or dermal, ethanol solution, 2 ml/kg

Observations and times:

- *Clinical signs:* daily
- *Dermal irritation:* daily
- *Body weights:* 2X/week
- *Food Consumption:* weekly
- *Gross pathology:* at sacrifice
- *Toxicokinetics:* Blood samples or tissue samples (spleen, thymus and mandibular, mesenteric, axillary and tracheobronchial lymph nodes) were obtained from animals at 4 (13 males/group) and 24 hours (12 males/group) after the last dose. ASM 981 levels were determined in the blood and tissue samples by _____
_____ The limit of quantification were ~ ng/ml for blood, – ng/g for lymph nodes, – ng/g for spleen and — ng/g for thymus.

Results:

- **Clinical signs** No treatment related effects on mortality or clinical signs were noted in this study.
- **Dermal Irritation** No dermal irritation was noted in this study.
- **Body weights** 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 gross pathology effects were noted in this study.
- **Toxicokinetics** The concentrations (mean \pm SD) of ASM 981 in blood (ng/ml) and tissue samples (ng/g) after either oral or dermal administration of 50 mg/kg/day ASM 981 for two weeks are provided in the following table.

Specimen	Oral		Dermal	
	4 hr Conc.	24 hr Conc.	4 hr Conc.	24 hr Conc.
Blood	363 \pm 122	18 \pm 17	235 \pm 109	20 \pm 6
Spleen	75 \pm 57	1.1 \pm 1.7	45 \pm 42	13 \pm 21
Thymus	160 \pm 177	2.6 \pm 2.7	177 \pm 97	130 \pm 108
Mandibular	986 \pm 302	99 \pm 25	2815 \pm 6.1	1320 \pm 372
Mesenteric	343 \pm 230	308 \pm 68	443 \pm 306	834 \pm 174
Axillary	1600 \pm 339	129 \pm 34	3029 \pm 483	2380 \pm 261
Tracheobronchial	1465	176	1549	664

Note: Tracheobronchial values have only one value that is composed of a pooled tissue sample. Therefore, there is no standard deviation.

The highest mean blood levels ASM 981 were found 4 hours after both oral and dermal administration (363 and 235 ng/ml, respectively). The blood levels were similar 24 hours post dose after oral and dermal administration (18 and 20 ng/ml, respectively). In most cases, the highest concentrations in the tissue samples were found at 4 hours post dose for either oral or dermal administration. The one exception for this is for the mesenteric lymph node after dermal administration where the 24 hour concentration was almost 2X greater than the 4 hour concentration. The measurable levels of ASM 981 were quite high in all of the lymph nodes 24 hours after dermal application. This is in contrast to ASM 981 levels noted in lymph nodes 24 hours after oral administration, which tended to be significantly less than the 4 hour level except for the mesenteric lymph node. Higher levels of ASM 981 were noted in mandibular, axillary and tracheobronchial lymph nodes compared to blood, spleen and thymus. The ratio of levels in these lymph nodes to blood levels was ~3-4X after oral administration and ~6-13X after dermal administration. The highest concentrations of ASM 981 were noted in mandibular and axillary lymph nodes after dermal administration.

Summary of individual study findings:

Oral or dermal administration of 50 mg/kg/day for 2 weeks did not cause any overt toxicity in mice in this study. All animals were exposed to ASM 981 in blood and selected tissue samples after oral or dermal administration. The highest ASM 981 concentrations were noted 4 hr after either oral or dermal administration except for mesenteric lymph nodes after dermal administration. Higher concentrations of ASM 981 were noted in lymph nodes compared to blood levels. The highest levels of ASM 981 were noted in mandibular and axillary lymph nodes after dermal administration. In general, high concentrations of ASM 981 are noted in lymph

nodes 24 hours after dermal application. This result suggests a preferred concentration of ASM 981 in these lymph nodes after dermal administration. The concentrations of ASM 981 in the thymus and spleen were lower or close to those noted in the blood.

Repeat Dose Toxicology Study #2:

A 13-week oral (per gavage) dose-range-finding study in mice (Drug form: lyophilisate suspension)

Study Title: A 13-week oral (per gavage) dose-range-finding study in mice (Drug form: lyophilisate suspension)
Study No: T-10/203-164
Sandoz Study No: 202DFM
Volume #, and page #: 13, 5-1
Conducting laboratory: Sandoz Pharma LTD, Basel, Switzerland
Date of study initiation: 1/3/95
GLP compliance: Yes
QA- Report: Yes (X) No ()
Drug, and lot#: ASM 981 – batch# 94903
Formulation/vehicle: Water & Plasmagelan^R (Note: ASM 981 lyophilisate was reconstituted using water and then further diluted with the plasma volume surrogate Plasmagelan^R to adjust to the final drug concentrations)

Methods:

Test article or vehicle (Plasmagelan^R) was administered orally (via gavage) on a daily basis, 7 days/week, for 13 weeks.

Dosing:

- *species/strain:* CD-1 mice
- *#/sex/group or time point:* 10/sex/dose
- *satellite groups used for toxicokinetics or recovery:* 9/sex/dose
- *age:* 8 weeks
- *weight:* males: 28 – 38 g; females: 23 – 30 g
- *doses in administered units:* 0, 10, 50, 100 and 312.5 mg/kg ASM 981
- *route, form, volume, and infusion rate:* oral; liquid suspension; 25 ml/kg/day

Observations and times:

- *Clinical signs:* daily
- *Body weights:* weekly
- *Food consumption:* weekly
- *Hematology:* during week 13
- *Clinical chemistry:* during week 13
- *Gross pathology:* at sacrifice
- *Organ weights:* brain, heart, kidneys, liver, testes

- *Histopathology:* The following organs were preserved in 10% buffered formalin from all animals: adrenals, aorta, bones (femur/knee/tibia, right), brain, esophagus, eyes, gall bladder, harderian glands, heart, intestine-small (duodenum, jejunum, ileum), intestine-large (cecum, colon, rectum), kidneys, larynx, liver, lungs, lymph nodes (mandibular, mesenteric, tracheobronchial), mammary glands, muscle (biceps femoris, right), nasal cavities, ovaries, pancreas, pituitary, prostate, salivary glands, sciatic nerve, seminal vesicles, skin, spinal cord, spleen, sternum, stomach, testes (with epididymides), thymus, thyroid (with parathyroids), tongue, trachea, urinary bladder, uterus (with cervix), vagina and all gross lesions.

All gross lesions from all animals were examined microscopically. All of the preserved tissues were examined from the control and high dose animals. In addition, the thymus, spleen, mesenteric lymph node, pancreas, stomach, small and large intestines, sternum, liver, ovaries, testes, uterus, vagina, prostate, esophagus and lung were examined microscopically in the remaining dose groups.

- *Toxicokinetics:* Blood samples were obtained from animals (2/sex/dose) at 0.5, 1, 2 and 4 hours after dosing on days 29 and 92. ASM 981 levels were determined in the blood samples. The limit of quantification was \sim ng/ml.

Results:

- **Clinical signs** No treatment related effects on mortality or clinical signs were noted in this study.
- **Body weights** 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.
- **Hematology** Mean absolute numbers of eosinophilic granulocytes were slightly increased in mid-low (\uparrow 1.5X) and mid-high dose males (\uparrow 1.9X) compared to control animals. Mean absolute numbers of eosinophilic granulocytes were slightly increased in low (\uparrow 1.8X), mid-low (\uparrow 2.4X) and high dose females (\uparrow 2.1X) compared to control animals.
- **Clinical Chemistry** Serum glucose was slightly increased in all treated males compared to control animals (range = \uparrow 1.3X – \uparrow 2.1X from low – high dose groups). Serum glucose was slightly increased in high dose females (\uparrow 1.3X) compared to control animals. Magnesium levels were

10	Male	1233	1109	1	0.5	2371	1028
	Female	2979	3480	0.5	0.5	2157	2949
50	Male	4602	6588	0.5	1	9368	15573
	Female	5377	5572	1	0.5	12168	14088
100	Male	5239	3814	1	1	14267	8618
	Female	10968	5800	1	1	22616	14743
312.5	Male	*	6738	*	1	*	**
	Female	*	14401	*	0.5	*	19929

* - Samples could not be re-analyzed due to a lack of volume

** - AUC could not be computed due to missing samples at 4 hours

A dose dependent increase in systemic exposure was noted in this study. It is difficult to draw any definitive conclusions based on $AUC_{0.5-4 \text{ hr}}$ values provided in the study report. It would have been more beneficial if $AUC_{0-\infty}$ values had been provided in the study report. C_{max} values were higher in female animals compared to male animals for all dose groups (except for the mid-low dose day 92 values). No indication of drug accumulation was noted in this study.

Summary of individual study findings:

Potential target organs of toxicity identified in this study included the pancreas, thymus, spleen/mesenteric lymph nodes and uterus/vagina. The effects noted in the thymus and spleen/mesenteric lymph nodes are probably related to the pharmacological (immunosuppressive) activity of ASM 981. The NOAEL identified in this study was 10 mg/kg/day ($AUC_{0.5-4 \text{ hr}} = 1029$ and 2949 ng·hg/ml in males and females, respectively) for mice after 13 weeks of oral administration of ASM 981.

Dermal Mouse FMF:

Repeat Dose Toxicology Study #3:

A 4-week dermal toxicokinetic study of SDZ ASM 981 cream administered to hairless mice

Study Title: A 4-week dermal toxicokinetic study of SDZ ASM 981 cream administered to hairless mice

Study No: T-12/203-197

Contract Study No: 972015

Volume #, and page #: 15, 5-1

Conducting laboratory: _____

Date of study initiation: 10/1/97

GLP compliance: Yes

QA- Report: Yes (X) No ()

Drug, and lot#: 0.6% ASM 981 cream – batch# Z045 0796
1.0% ASM 981 cream – batch# Z045 0896

Formulation/vehicle: Vehicle cream – batch# Z068 0896

9; 0.6; 0.03	Male	102	8	1360
	Female	134	8	1780
15; 0.6; 0.05	Male	157	8	2150
	Female	178	8	2470
15; 1.0; 0.03	Male	113	8	1630
	Female	200	8	2570
25; 1.0; 0.05	Male	144	8	1950
	Female	335	8	4250

Peak drug concentrations were noted on day 26 at 8 hours post dose. In general, there appeared to be a dose dependent increase in systemic exposure. The systemic exposure for both 15 mg/kg/day dose groups was approximately equivalent. Female mice had consistently higher C_{max} and AUC values compared to male mice.

Summary of individual study findings:

Slight erythema, edema and flaking were noted in male and female mice in all test article and vehicle formulation groups. Animals in all ASM 981 cream treatment groups were exposed to compound in the blood. In general, there appeared to be a dose dependent increase in systemic exposure.

Repeat Dose Toxicology Study #4:

Range-finding tolerance test of SDZ ASM 981 administered topically to hairless mice for 8 weeks

Study Title: Range-finding tolerance test of SDZ ASM 981 administered topically to hairless mice for 8 weeks

Study No: T-13/203-189

Contract Study No: 1014-001P

Volume #, and page #: 15, 5-162

Conducting laboratory: _____

Date of study initiation: 10/1/97

GLP compliance: Yes

QA- Report: Yes (X) No ()

Drug, and lot#: 0.2% ASM 981 cream – batch# Z042 0796
0.6% ASM 981 cream – batch# Z045 0796
1.0% ASM 981 cream – batch# Z061 0896

Formulation/vehicle: Vehicle cream – batch# Z068 0896

Methods:

This study consisted of two parts. Part I was designed to assess the phototoxicity or photoprotective effects of the test article formulations. Part II was an 8 week range finding tolerance test to determine the most appropriate concentrations of the test article formulations to be use in a photocarcinogenesis study.

In Part I of this study, mice were lightly anesthetized and positioned on trays with masking tape. Test article (100 µl) was applied topically. The duration of time between test article administration and UVR exposure was approximately 15 min except for 8-methoxypsoralen (8-MOP) which was treated 1 hour prior to UVR exposure. An aluminum foil mask was placed over each animal before UVR exposure. The mask had six holes, each with a diameter of 4 mm that served to define the irradiation sites. A single exposure of UVR was given to each of the irradiation sites. The radiant intensity of the source was checked at regular interval with a meter. A positive control (8-methoxypsoralen), negative control (sunscreen) and untreated control were incorporated into the study design.

Study Design – Part I

Treatment	UVR Exposure (MedD) ^a					
	0	0.5	1.0	1.4	2.0	2.7
Vehicle cream	+	+	+	+	+	+
0.2% ASM 981 cream	+	+	+	+	+	+
0.6% ASM 981 cream	+	+	+	+	+	+
1.0% ASM 981 cream	+	+	+	+	+	+
8-MOP ^b	+	+	+	N/A ^c	N/A ^c	N/A ^c
SPF4 SS ^b	+	+	+	+	+	+
Untreated	+	+	+	+	+	N/A ^c

- a: MEDD refers to a UV dose adequate to elicit a barely perceptible response in skin; the range of doses delivered was thus 0 to 2.7 times MEDD.
- b: 8-MOP: 8-methoxypsoralen in methanol (1 mg/ml): SFP 4 SS: Sun protection factor 4 sunscreen
- c: Not applicable

In Part II of the study, test article treated mice received 100 µl of various test article formulations (0, 0.2% 0.6% and 1.0% ASM 981 cream). In addition, an untreated control was included in the study. Test article was applied for 5 days/week, for 8 weeks and remained on the skin for 24 hours. The sequence of irradiation and test article application alternated from one irradiation day to the next. On Monday, Wednesday and Friday irradiation occurred after test article application and on Tuesday and Thursday the irradiation preceded test article application. There was ~60 min between dosing of the animals and UV irradiation or between UV irradiation and dosing of the animals. The level of exposure UVR (600 RBU/week) equaled that typically used in a photocarcinogenicity study. Light intensity was monitored on representative racks by dosimeters.

Dosing:

- *species/strain*: albino hairless Crl:SkH1-hrBR mice
- *#/sex/group or time point*: 3/sex/group
- *satellite groups used for toxicokinetics or recovery*: NA
- *age*: 5 weeks
- *weight*: 15 – 22 grams

- *doses in administered units:* Part I – 0 (0%), 10 (0.2%), 30 (0.6%) and 50 (1.0%) mg/kg/day ASM 981; positive (8-MOP), negative (SS) and untreated controls were included
Part II – untreated control and 0 (0%), 10 (0.2%), 30 (0.6%) and 50 (1.0%) mg/kg/day ASM 981
- *route, form, volume, and infusion rate:* topical; cream; 0.10 ml/mouse
- *source of irradiation (part I):* — : compact arch high intensity solar simulator —
- *type of filter (part I & II):* 1 mm — glass filter
- *source of irradiation (part II):* 6.5 KW xenon log arc water cooled burner —

Observations and times:

- *Clinical signs:* Part II – weekly
- *Local dermal signs:* Part I – 24, 48 and 72 hours after irritation; Part II – weekly
- *Body weights:* Part II - weekly
- *Gross pathology:* Part II – at sacrifice
- *Histopathology:* Part II – Samples of skin from the site of administration (~1.0 x 0.5 cm) or an equivalent area on untreated mice were processed for histopathological analysis.

Results (Part I):

- **Clinical signs** No mortality was noted in this study. No treatment related clinical signs were noted in this study.
- **Local dermal signs** The anticipated calculated mean MEdD value in naïve skin at 48 hours after UVR exposure was 1.0 (i.e., a 1.0 mean MEdD value would indicate that neither a photoprotective nor a phototoxic effected occurred). The calculated MEdD (mean \pm SD) for the vehicle, low, mid and high dose groups were 1.1 ± 0.2 , 1.1 ± 0.2 , 1.1 ± 0.2 , 1.1 ± 0.2 and 1.3 ± 0.2 , respectively. The positive (8-MOP; MEdD ≤ 0.5) and negative (SPF 4 SS; MEdD >2.7) controls yielded appropriate responses in this study.

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Results (Part II):

- **Clinical signs** No mortality was noted in this study. No treatment related clinical signs were noted in this study.
- **Local dermal signs** Grade 1 erythema and grade 1 flaking was noted in all vehicle and ASM 981 cream treatment groups. No dermal effects were noted in the skin of untreated mice.
- **Body weights** No treatment related effects on body weight were noted in this study.
- **Gross pathology** No treatment related gross pathology effects were noted in this study.
- **Histopathology** Mild to marked acanthosis/hyperkeratosis of the epidermis, minimal or mild hyperplasia of sebaceous glands and variable amounts of dermal inflammatory cell infiltrations ranging from a minimal to moderate amount were noted in skin samples obtained from all vehicle and ASM 981 cream treatment groups. No cutaneous changes were noted in the skin of untreated mice.

Summary of individual study findings:

The results of Part I of this study indicate that ASM 981 cream up to 1.0% was neither phototoxic or photoprotective under the conditions of this study.

Mild indicators of dermal irritation (with corresponding histopathological indications in the skin) were noted in all vehicle and ASM 981 cream treatment groups in Part II of this study. Based on this result the contract lab recommended that the vehicle and 0.2%, 0.6% and 1.0% ASM 981 cream formulations would be reasonably tolerated in a photocarcinogenesis study.

Dermal Mouse non-FMF:**Repeat Dose Toxicology Study #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)

Study Title: Preliminary toxicity study by dermal administration to CD-1 mice for 13 weeks followed by a 4 week reversibility period (Drug form: Ethanol solution)

Study No: T-19/203-136

Contract Study No: SPM067/0248

Volume #, and page #: 18, 5-1

Conducting laboratory: _____

Date of study initiation: 9/5/95

GLP compliance: Yes

QA- Report: Yes (X) No ()
Drug, and lot#: ASM 981 – batch# 95907
Formulation/vehicle: Ethanol

Methods:

Approximately 24 hours before treatment commenced, the dorsum between the limb girdles was clipped free of hair using electric clippers. The clipped area was approximately 10% of the total body surface area. Clipping was repeated on an as needed basis. Animals were treated daily with 0.05 ml of vehicle or test article formulations that was distributed evenly over the clipped area. The test site was washed 24 hours after administration each day to remove any test material residue prior to the next dosing. All animals were dosed once each day, 7 days/week, for a duration of 13 weeks. Assigned animals from untreated, control and high dose groups received treatment for 13 weeks followed by a 4 week recovery period.

Dosing:

- *species/strain:* CD-1 mice
- *#/sex/group or time point:* refer to study design table below
- *satellite groups used for toxicokinetics or recovery:* refer to study design table below
- *age:* 35 – 42 days
- *weight:* males: 26 – 33 g; females: 21 – 26 g
- *doses in administered units:* refer to study design table below
- *route, form, volume, and infusion rate:* topical; ethanol solution; 0.05 ml/day

Study Design

Dose (mg/kg)*	Stock Sol. Conc. (mg/ml)	Number of Main Study Animals		Number of Recovery Animals		Number of Toxicokinetic Animals	
		Male	Female	Male	Female	Male	Female
0 (Untreated)	0	10	10	5	5	--	--
0 (Vehicle)	0	10	10	5	5	6	6
6	3	10	10	--	--	6	6
60	30	10	10	--	--	6	6
600	300	10	10	5	5	6	6

* - Dose (mg/kg) estimated based on the weight of a mouse equal to 25 grams

Observations and times:

- *Clinical signs:* daily
- *Body weights:* weekly
- *Food consumption:* weekly
- *Hematology:* during week 13 and 17 (for recovery animals)
- *Clinical chemistry:* during week 13 and 17 (for recovery animals)
- *Urinalysis:* during week 13 and 17 (for recovery animals)

- *Gross pathology:* at sacrifice
- *Organ weights:* brain, epididymides, heart, kidneys, liver, lungs, ovaries, prostate, salivary glands (submandibular), seminal vesicles, spleen, testes, thymus and uterus (with cervix)
- *Histopathology:* The following organs were preserved in 10% buffered formalin from all animals: adrenals, aorta, brain, bronchi, cecum, colon, duodenum, epididymides, esophagus, eyes, femoral bone (and marrow), gall bladder, heart, ileum, jejunum, kidneys, lachrymal glands, liver, lungs, lymph nodes (mandibular, mesenteric), mammary glands, ovaries, pancreas, pituitary, prostate, rectum, salivary glands (submandibular), sciatic nerve, seminal vesicles, skeletal muscle (left thigh), skin (treated and untreated), spinal cord, spleen, sternum, stomach, testes, thymus, thyroid (with parathyroids), tongue, trachea, urinary bladder, uterus (with cervix), vagina and all gross lesions.

All gross lesions from all animals were examined microscopically. The following preserved tissues were examined from the vehicle control and high dose animals: Adrenals, brain, eyes, femur, heart, kidneys, liver, lungs, mandibular and mesenteric lymph nodes, pancreas, skin (treated and untreated), spinal cord, sternum, stomach, thyroid and uterus. In addition, the thymus, spleen, kidneys, mandibular and mesenteric lymph nodes, ovaries, uterus and salivary glands were examined microscopically in the remaining dose groups and recovery animals.

- *Toxicokinetics:* Blood samples were obtained from animals (3/sex/dose) at 6 and 24 hours after dosing on day 91. ASM 981 levels were determined in the blood samples by _____

_____ The limit of quantification was ~ ng/ml.

Results:

- **Clinical signs** Clinical signs noted in high dose animals included piloerection, hunched posture, underactivity or overactivity, thin build, pallor, hypothermia, partially closed eyelids, abnormal respiration and yellow perigenital staining. Two males and five females were killed *in extremis* in the high dose group due to the severity of the clinical signs (sacrificed between weeks 8 – 13). The clinical signs noted in high dose animals reduced during the 4 week recovery period
- **Body weights** Overall bodyweight gain was reduced by 34% in high dose males compared to vehicle control males. Body weight gain in high dose

males returned to levels comparable to vehicle control males during the 4 week recovery period.

- **Food Consumption** No treatment related effects on food consumption were noted in this study.
- **Hematology** Reduced mean lymphocyte counts were noted in high dose males ($\downarrow 45\%$) and mid ($\downarrow 41\%$) and high dose females ($\downarrow 34\%$) compared to vehicle control animals. Eosinophil counts were significantly higher in high dose male ($\uparrow 4X$) and female ($\uparrow 3X$) animals compared to vehicle control animals. Eosinophil counts remained higher in high dose male ($\uparrow 3X$) and female ($\uparrow 2X$) animals at the end of the recovery period compared to vehicle control animals.
- **Clinical Chemistry** Plasma magnesium concentrations were decreased in mid (males: $\downarrow 18\%$; females: $\downarrow 20\%$) and high dose animals (males: $\downarrow 31\%$; females: $\downarrow 35$) compared to vehicle control animals. Plasma alkaline phosphatase activities were increased in mid dose females ($\uparrow 1.4X$) and high dose animals (males: $\uparrow 1.9X$; females: $\uparrow 1.4X$) compared to vehicle control animals. Plasma glucose levels were increased in high dose animals (males: $\uparrow 2.4X$; females: $\uparrow 1.5X$) compared to vehicle control animals. Clinical chemistry values returned to normal at the end of the recovery period.
- **Urinalysis** Increased urine volume was noted in high dose animals (males: $\uparrow 2.6X$; females: $\uparrow 1.6X$) compared to vehicle control animals. A lower pH was noted in high dose males ($\downarrow 11\%$) compared to vehicle control animals.
- **Gross pathology** A high incidence of pale untreated skin was noted in high dose males at the 13 week necropsy. This finding was not apparent in high dose males at the 17 week (recovery group) necropsy.
- **Organ weights** Absolute liver weight was increased in high dose females ($\uparrow 1.2X$) compared to vehicle control females. Absolute submandibular salivary gland weight was decreased in high dose males ($\downarrow 45\%$) and females ($\downarrow 14\%$) compared to vehicle control animals. Absolute seminal vesicle weight was reduced in high dose males ($\downarrow 33\%$) compared to vehicle control males. Absolute uterus + cervix weight was reduced in high dose females ($\downarrow 41\%$) compared to vehicle control females. Absolute uterus + cervix weight remained reduced in high dose females ($\downarrow 25\%$) at the end of the recovery period compared to vehicle control females.

- **Histopathology** Treatment related findings were noted in the hemopoietic tissue, mandibular and mesenteric lymph nodes, spleen, thymus, pancreas, ovaries, uterus or cervix, kidneys and salivary glands.

Hemopoietic tissue:

Pleomorphic lymphoma was noted in 2/10 mid dose males and 1/10 mid dose females. Pleomorphic lymphoma was also noted in one high dose female that was killed *in extremis* during the treatment period.

Mandibular lymph node:

Pleomorphic lymphoid cell proliferation was noted in mid (males: 2/10; females: 1/9) and high dose animals (males: 1/8; females: 1/5). Sinus histiocytosis was noted in 1/9 mid dose females and 1/8 high dose males and 2/5 high dose females.

Mesenteric Lymph Node:

An increased incidence of lymphocytolysis was noted in mid dose animals (males: 8/10; females: 6/10) compared to vehicle control animals (males: 1/10; females: 1/10). Pleomorphic lymphoid cell proliferation was noted in 1/10 mid dose males and 1/10 mid dose females. Pleomorphic lymphoid cell proliferation was also noted in one high dose female that was killed *in extremis* during the treatment period.

Spleen:

Lymphocytolysis was noted in two high dose males and one high dose female that were killed *in extremis* during the treatment period. Pleomorphic lymphoid cell proliferation was noted in 1/10 mid dose males and one high dose male and one high dose female killed *in extremis* during the treatment period.

Thymus:

Medullary atrophy was noted in mid (male: 3/10; females: 6/10) and high dose animals (males: 6/8; females: 5/6). Medullary atrophy was also noted in high dose males and four high dose females killed *in extremis* during the treatment period. Cortical lymphoid hyperplasia was noted in 1/10 mid dose females and 1/8 high dose males and 2/6 high dose females. Pleomorphic lymphoid cell proliferation was noted in 1/10 mid dose males.

Pancreas:

Minimal vacuolation of the islets were noted in 3/6 high dose females.

Ovaries:

Arrested follicular development was noted in 3/10 mid dose females and 3/6 high dose females. Arrested follicular development was also noted in three high dose females killed *in extremis* during the treatment period.

Uterus/cervix:

Atrophy in the uterus or cervix was noted in 1/10 mid dose females and 3/6 high dose females. Atrophy was also noted in all five of the high dose females killed *in extremis* during the treatment period. The study report states that this change was probably associated with the low uterus weights noted at necropsy for high dose females.

Kidneys:

Basophilic cortical tubules were noted in mid (males: 4/10; females: 2/10) and high dose animals (males: 2/8; females: 3/6). Cortical lymphocytic infiltration was noted in 2/10 mid dose males and 2/10 mid dose females and 3/6 high dose females. Chronic interstitial nephritis was noted in 1/10 mid dose males and 2/10 mid dose females and 1/6 high dose females.

Salivary glands:

Degranulation of the granular convoluted ducts was noted in high dose animals (males: 4/8; females: 3/6). The study report states that this correlated with the reduced submandibular salivary gland weight noted at the 13 week necropsy.

Recovery phase histopathology findings:

Pleomorphic lymphoma was not noted in animals killed on completion of the recovery period. Findings noted in the mandibular and mesenteric lymph nodes, ovaries and kidneys noted after 13 weeks of treatment were not clearly reversible after four weeks without treatment. This suggests that rapid recovery from the changes in these organs can not be expected for ASM 981 treatment.

- **Toxicokinetics** A summary of the blood concentrations (mean \pm SD) of ASM 981 after 13 weeks of dermal administration in mice is provided in the following table.

Dose (mg/kg/day)	Time (hour)	Male Conc. (ng)	Female Conc. (ng)
6	6	77 \pm 19	92 \pm 25
	24	26 \pm 5	31 \pm 8
60	6	68 \pm 44	101 \pm 24
	24	58 \pm 5	54 \pm 3
600	6	662 \pm 286	947 \pm 397
	24	101 \pm 30	277 \pm 111

ASM 981 was observed in the blood of animals in all ASM 981 dose groups at 6 and 24 hours after the last dermal application of ASM 981 after 13 weeks of treatment. A dose dependent increase was noted between the mid and high dose groups. However, the low and mid dose groups showed approximately equivalent blood concentrations. Blood concentrations of ASM 981 were higher at 6 hours vs 24 hours and appeared to be higher in females compared to males.

Summary of individual study findings:

Potential target organs of toxicity identified in this study included the hemopoietic tissue, mandibular and mesenteric lymph nodes, spleen, thymus, ovaries, uterus or cervix, kidneys and salivary glands. The effects noted in the hemopoietic tissue, mandibular and mesenteric lymph nodes, spleen and thymus are probably related to the pharmacological (immunosuppressive) activity of ASM 981. The effects noted in the pancreas, ovaries, uterus or cervix, kidneys and salivary glands are probably related to the overt toxicological properties of ASM 981. The NOAEL identified in this study was 6 mg/kg/day for mice after 13 weeks of topical administration of ASM 981 dissolved in ethanol.

It is interesting to note that the sponsor states the following in the study report. In view of the pleomorphic lymphomas observed at the concentration of 30 mg/ml (60 mg/kg/day) that may be related to the pharmacological action of the test material, the concentration of 4 mg/ml (8 mg/kg/day) was selected as the highest dose in the forthcoming oncogenicity study. It would have been more beneficial if the sponsor had selected a dose where pleomorphic lymphomas may have been observed in the oncogenicity study to provide better data for the analysis of risk to humans. However, the sponsor did not submit the study protocol for the dermal mouse carcinogenicity study to the agency for analysis prior to initiation of this study. The sponsor initiated this study without the knowledge of the agency.

Repeat Dose Toxicology Study #6:

Study to investigate the dosage response of immunosuppression and lymphoproliferative disorders following dermal administration to CD-1 mice for 13 weeks

<u>Study Title:</u>	Study to investigate the dosage response of immunosuppression and lymphoproliferative disorders following dermal administration to CD-1 mice for 13 weeks
<u>Study No:</u>	T-89/203-181
<u>Contract Study No:</u>	SAZ 579/970388
<u>Volume #, and page #:</u>	50, 5-1
<u>Conducting laboratory:</u>	_____
<u>Date of study initiation:</u>	12/13/96
<u>GLP compliance:</u>	Yes
<u>QA- Report:</u>	Yes (X) No ()
<u>Drug, and lot#:</u>	ASM 981 – batch# 95912
<u>Formulation/vehicle:</u>	Ethanol

Methods:

Approximately 24 hours before treatment commenced, the dorsum between the limb girdles was clipped free of hair using electric clippers. The clipped area was approximately 10% of the total body surface area. Clipping was repeated on an as needed basis. Animals were treated daily with 2 ml/kg of vehicle or test article formulations that was distributed evenly over the clipped area. The test site was washed 24 hours after administration each day to remove any test material residue prior to the next dosing. All animals were dosed once each day, 7 days/week, for a duration of 13 weeks.

Dosing:

- *species/strain*: CD-1 mice
- *#/sex/group or time point*: 25/sex/dose
- *satellite groups used for toxicokinetics or recovery*: N/A
- *age*: 21 - 28 days
- *weight*: males: 22 - 28 g; females: 20 - 27 g
- *doses in administered units*: 0 (ethanol), 0.1, 0.5, 1, 5, 10, 25 and 50 mg/kg/day
- *route, form, volume, and infusion rate*: topical; ethanol solution; 2 ml/kg

Observations and times:

- *Clinical signs*: daily
- *Body weights*: weekly
- *Food consumption*: weekly
- *Hematology*: during week 13 from 10/sex/dose
- *Gross pathology*: at sacrifice
- *Organ weights*: spleen and thymus
- *Histopathology*: The following organs were preserved for histological analysis from all treated animals: lymph nodes (axillary, mandibular, mesenteric, tracheobronchial), spleen and thymus.

- *Toxicokinetics*: Blood samples were obtained from animals (5/sex/dose) at 1, 2, 4, 7 and 24 hours after dosing during weeks 4 and 12 of treatment. ASM 981 levels were determined in the blood samples by _____ The limit of quantification was _____ ng/ml.

Results:

- **Clinical signs** A higher incidence of piloerection was noted during the first six weeks of treatment in all ASM 981 dose groups. Females were not similarly affected by ASM 981 treatment. No treatment related mortality was noted in this study.

- **Body weights** 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.
- **Hematology** No treatment related effects on hematology parameters were noted in this study.
- **Gross pathology** Enlarged axillary lymph nodes were noted in 6/25 high dose females.
- **Organ weights** Absolute spleen weight was slightly increased in high dose males ($\uparrow 1.2X$) and high dose females ($\uparrow 1.1X$) compared to vehicle control animals.
- **Histopathology** Treatment related findings were noted in the hemopoietic tissue, thymus, spleen and axillary, mandibular and mesenteric lymph nodes.

Hemopoietic tissue:

Pleomorphic lymphoma was noted in one male in the 25 mg/kg/day dose group and one male in the 50 mg/kg/day dose group. Pleomorphic lymphoid proliferation was noted in one male in the 25 mg/kg/day dose group and 3 males and one female in the 50 mg/kg/day dose group.

Thymus:

Minimal/mild medullary atrophy was noted in 25 mg/kg/day animals (male: 13/25; females: 9/25) and 50 mg/kg/day animals (male: 17/25; females: 12/25). Minimal/mild cortical hyperplasia was noted in 25 mg/kg/day animals (male: 6/25; females: 9/25) and 50 mg/kg/day animals (male: 6/25; females: 12/25).

Spleen:

Minimal/moderate lymphoid hyperplasia was noted in 25 mg/kg/day animals (male: 7/25; females: 9/25) and 50 mg/kg/day animals (male: 10/25; females: 7/25).

Axillary lymph node:

Minimal/moderate lymphoid hyperplasia was noted in 7/25 females at 50 mg/kg/day.

Mandibular lymph node:

Minimal/moderate lymphoid hyperplasia was noted in 11/25 males at 25 mg/kg/day and 50 mg/kg/day animals (male: 8/25; females: 12/25).

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Mesenteric Lymph Node:

Minimal lymphoid hyperplasia was noted in 50 mg/kg/day animals (male: 6/25; females: 10/25).

- **Toxicokinetics** A summary of the toxicokinetic (mean) parameters is provided in the following table.

Dose (mg/kg/day)	Sex	C_{max} (ng/ml)		T_{max} (hr)		$AUC_{0-24\text{ hr}}$ (ng·hg/ml)	
		Week 4	Week 12	Week 4	Week 12	Week 4	Week 12
0.1	Male	8.8	5.9	7	1	134	94.2
	Female	10.0	5.0	1	2	106	64.7
0.5	Male	14.8	15.0	4	7	229	255
	Female	27.5	21.7	4	4	422	256
1	Male	23.4	16.8	4	7	328	307
	Female	44.4	25.1	4	4	683	358
5	Male	33.3	29.4	4	7	535	517
	Female	54.3	31.4	4	7	805	515
10	Male	57.0	47.5	2	2	717	643
	Female	47.2	43.2	4	4	644	675
25	Male	125	285	1	2	1650	1854
	Female	414	465	2	1	2614	1745
50	Male	477	449	2	2	5725	4703
	Female	630	614	2	2	6077	4200

A dose dependent increase in systemic exposure was noted in this study. No indication of drug accumulation was noted in this study. No apparent difference in systemic exposure based on sex was noted in this study.

Summary of individual study findings:

The focus of this study was to assess the dose response relationship of immunosuppression and lymphoproliferative disorders following dermal administration of ASM 981. Potential target organs of toxicity identified in this study included the hemopoietic tissue, thymus, spleen and axillary and mandibular and mesenteric lymph nodes. Doses of 25 and 50 mg/kg/day by dermal administration for 13 weeks were associated with lymphoproliferative changes, including malignancies. These findings were generally dose related in incidence and severity. No lymphoproliferative changes were noted at the 10 mg/kg/day dose level. Therefore, 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.

Repeat Dose Toxicology Study #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

Study Title: 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

Study No: T-90/203-192

Contract Study No: SAZ 583/971946

Volume #, and page #: 52, 5-1

Conducting laboratory: _____

Date of study initiation: 1/25/97

GLP compliance: Yes

QA- Report: Yes (X) No ()

Drug, and lot#: ASM 981 – batch# 95912

Formulation/vehicle: Ethanol

Methods:

Approximately 24 hours before treatment commenced, the dorsum between the limb girdles was clipped free of hair using electric clippers. The clipped area was approximately 10% of the total body surface area. Clipping was repeated on an as needed basis. Animals were treated daily with 2 ml/kg of vehicle or test article formulations that was distributed evenly over the clipped area. The test site was washed 24 hours after administration each day to remove any test material residue prior to the next dosing. All animals were dosed once each day, 7 days/week, for a duration of either 4 weeks (25/sex/dose), 8 weeks (25/sex/dose) or 13 weeks (25/sex/dose).

Dosing:

- *species/strain:* CD-1 mice
- *#/sex/group or time point:* 75/sex/dose (Note: 25/sex/dose were sacrificed after 4, 8 and 13 weeks, respectively)
- *satellite groups used for toxicokinetics or recovery:* N/A
- *age:* 35 - 42 days
- *weight:* males: 23.7 – 30.9 g; females: 19.4 – 26.8 g
- *doses in administered units:* 0 (ethanol), 25, 50, 100 and 200 mg/kg/day
- *route, form, volume, and infusion rate:* topical; ethanol solution; 2 ml/kg

Observations and times:

- *Clinical signs:* daily
- *Body weights:* weekly
- *Food consumption:* weekly
- *Hematology:* at necropsy at week 4, 8 or 13 of treatment
- *Gross pathology:* at necropsy at week 4, 8 or 13 of treatment
- *Organ weights:* spleen and thymus (for weeks 4, 8 or 13)

- *Histopathology:* The following organs were preserved for histological analysis from all treated animals (for weeks 4, 8 or 13): lymph nodes (axillary, mandibular, mesenteric, trancheobronchial), spleen and thymus.
- *Toxicokinetics:* Blood samples were obtained from animals (5/sex/dose) at 1, 2, 4, 7 and 24 hours after dosing during weeks 4, 8 or 13 of treatment. ASM 981 levels were determined in the blood samples by _____ The limit of quantification was — ng/ml.

Results:

- **Clinical signs** A higher incidence of piloerection was noted in all ASM 981 treated groups of males and females in the mid-low, mid-high and high dose groups. A higher incidence of overactivity was noted in high dose females only. No treatment related mortality was noted in this study.
- **Body weights** 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.
- **Hematology** No treatment related effects on hematology parameters were noted in this study.
- **Gross pathology** Swollen and mottled spleen was noted at higher incidences in high dose males after 8 weeks compared to control animals. Swollen spleen was noted at higher incidences in all ASM 981 treated males and mid-low, mid-high and high dose females at 13 weeks compared to control animals. In addition, mottled spleen was noted at higher incidences in mid-high and high dose males and high dose females at 13 weeks compared to control animals.
- **Organ weights** Absolute spleen weight was slightly increased after 8 weeks and 13 weeks in mid-high (8 weeks: ↑1.3X ; 13 weeks: ↑1.2X) and high dose males (8 weeks: ↑1.3X ; 13 weeks: ↑1.2X) compared to vehicle control animals.

Absolute thymus weight was slightly increased after 4 week, 8 weeks and 13 weeks in mid-high (4 weeks: ↑1.1X; 8 weeks: ↑1.1X ; 13 weeks: ↑1.2X) and high dose females (4 weeks: ↑1.1X; 8 weeks: ↑1.2X ; 13 weeks: ↑1.4X) compared to vehicle control animals.

- **Histopathology** Treatment related findings were noted in the hemopoietic tissue, thymus and spleen.

Hemopoietic tissue:

Pleomorphic lymphoid proliferation was noted in 1/25 mid-high dose males after 4 weeks of treatment. Pleomorphic lymphoid proliferation was noted in 2/25 mid-low dose males, 1/25 mid-high dose males, 4/25 high dose males and 2/25 high dose females after 8 weeks of treatment. Pleomorphic lymphoid proliferation was noted in 1/25 low dose males, 1/25 mid-low dose males, 1/25 mid-low dose females, 6/25 mid-high dose males, 4/25 high dose males and 5/25 high dose females after 13 weeks of treatment.

Pleomorphic malignant lymphoma was noted in 1/25 mid-high dose males and 1/25 high dose females after 8 weeks of treatment. Pleomorphic malignant lymphoma was noted in 1/25 mid-low dose females, 1/25 mid-high dose males and 2/25 high dose females after 13 weeks of treatment.

Thymus:

Medullary atrophy was noted in mid-low dose animals (males: 17/25; females: 10/25), mid-high dose animals (males: 24/25; females: 18/25) and high dose animals (males: 23/25; females: 22/25) after 4 weeks of treatment. Medullary atrophy was noted in low dose males (9/25), mid-low dose animals (males: 8/25; females: 7/25), mid-high dose animals (males: 20/25; females: 23/25) and high dose animals (males: 18/25; females: 23/25) after 8 weeks of treatment. Medullary atrophy was noted in mid-low dose females (7/25), mid-high dose animals (males: 11/25; females: 15/25) and high dose animals (males: 19/25; females: 21/25) after 13 weeks of treatment.

Cortical hyperplasia was noted in low dose animals (males: 7/25; females: 8/25), mid-low dose animals (males: 16/25; females: 10/25), mid-high dose animals (males: 17/25; females: 17/25) and high dose animals (males: 15/25; females: 22/25) after 4 weeks of treatment. Cortical hyperplasia was noted in low dose animals (males: 13/25; females: 11/25), mid-low dose animals (males: 13/25; females: 17/25), mid-high dose animals (males: 16/25; females: 24/25) and high dose animals (males: 13/25; females: 24/25) after 8 weeks of treatment. Cortical hyperplasia was noted in mid-low dose animals (males: 8/25; females: 15/25), mid-high dose animals (males: 11/25; females: 17/25) and high dose animals (males: 16/25; females: 18/25) after 13 weeks of treatment.

Spleen:

Lymphoid atrophy was noted in low dose animals (males: 3/25; females: 4/25), mid-low dose animals (males: 11/25; females: 2/25), mid-high dose animals (males: 9/25; females: 7/25) and high dose animals (males: 11/25; females: 8/25) after 4 weeks of treatment only.