- **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)			$AUC_{0\text{-}24\ hr}$ (ng·hr/ml)		
		Week 4	Week 8	Week 13	Week 4	Week 8	Week 13	Week 4	Week 8	Week 13
25	Male	143	160	86	1	2	1	1568	1829	1252
	Female	172	197	270	2	2	1	1704	1738	1463
50	Male	567	283	458	2	7	1	3090	4177	5075
	Female	410	528	520	1	2	2	4240	4587	3379
100	Male	896	748	568	2	2	2	8470	6828	6253
	Female	891	927	794	1	2	1	8616	8260	8500
200	Male	1057	1088	820	2	1	2	9636	8507	8291
	Female	1129	1455	1113	1	2	2	12997	14640	12546

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 severity of immunosuppression and the rate of onset of lymphoproliferative disorders following dermal administration of ASM 981 to CD-1 mice. Potential target organs of toxicity identified in this study included the hemopoietic tissue, thymus and spleen. Doses of 25 mg/kg/day and above by dermal administration for 13 weeks were associated with lymphoproliferative changes indicative of immunossuppression, including malignancies. These findings were generally dose related in incidence and severity. No NOAEL was established in this study. Pleomorphic lymphoma was noted in the mid-high (100 mg/kg/day) and high dose (200 mg/kg/day) groups after 8 weeks of treatment. No pleomorphic lymphoma was noted in the low dose group (25 mg/kg/day) after 13 weeks of treatment. The results of this study indicate that at the proper dose level (100 mg/kg/day or greater) pleomorphic lymphoma can be noted as early as after 8 weeks of treatment.

Repeat Dose Toxicology Study #8:

Investigational dermal painting study in mice: 1, 2, 3-month treatment with a 13 week recovery period

Study Title:	Investigational dermal painting study in mice: 1, 2, 3-month treatment with a 13 week recovery period
Study No:	T-91/203-207
Contract Study No:	15049 TCS
Volume #, and page #:	55, 5-1
Conducting laboratory:	[redacted]
Date of study initiation:	12/17/96
GLP compliance:	Yes
QA- Report:	Yes (X) No ()
Drug, and lot#:	ASM 981 – batch# 96914

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 40 – 50 µl of vehicle or test article formulation that was distributed evenly over the clipped area. The test site was not washed and not occluded during the treatment period. All animals were dosed once each day, 7 days/week, for a duration of either 4 weeks (40/sex/dose), 9 weeks (40/sex/dose) or 13 weeks (40/sex/dose). For each treatment period, half of the treated animals were sacrificed after the treatment period (20/sex/dose) and the other half were kept for a 13 week recovery phase (20/sex/dose).

Dosing:

- *species/strain:* CD-1 mice
- *#/sex/group or time point:* 40/sex/dose/treatment duration (4, 9 or 13 weeks)
- *satellite groups used for toxicokinetics or recovery:* 20/sex/dose for 13 week recovery
- *age:* 8 weeks
- *weight:* males: 27.2 – 37.4 g; females: 21.8 – 30.0 g
- *doses in administered units:* 0 (ethanol) and 100 mg/kg/day
- *route, form, volume, and infusion rate:* topical; ethanol solution; 40 – 50 µl/mouse

Observations and times:

- *Clinical signs:* daily
- *Body weights:* weekly
- *Food consumption:* weekly
- *Hematology:* performed at the end of each treatment period and recovery period
- *Gross pathology:* at necropsy at the end of each treatment period and recovery period
- *Organ weights:* spleen and thymus (at the end of each treatment period and recovery period)
- *Histopathology:* The following organs were preserved for histological analysis from all treated animals (at the end of each treatment period and recovery period): lymph nodes (cervical, mandibular, mesenteric, thoracic), spleen and thymus.

Results:

- **Clinical signs** No treatment related effects on clinical signs or 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** No treatment related effects on gross pathology were noted in this study.

- **Organ weights** Absolute thymus weight was significantly decreased after 9 weeks in treated females (↓25%) compared to vehicle control females. This effect was no longer apparent after the 13 week recovery period. No other significant effects on organ weights were noted in this study.

- **Histopathology** Minimal/mild thymic medullary atrophy was noted in ASM 981 treated animals after 4 weeks (males: 19/20; females: 13/20), 9 weeks (males: 13/20; females: 13/20) and 13 weeks of treatment (males: 9/20; females: 15/20). This treatment related finding was not noted in any of recovery groups for the 3 treatment duration periods.

  Pleomorphic lymphoid proliferation was noted in 1/20 ASM 981 treated males after 9 weeks of treatment. No pleomorphic lymphoid proliferation was noted in the recovery group for the 9 week treatment duration. Pleomorphic lymphoid proliferation was noted in 2/20 ASM 981 treated males in the recovery group for the 13 week duration of treatment. It is interesting to note that pleomorphic lymphoid proliferation was not noted in the 13 week treatment group.

Summary of individual study findings:

The findings from this study correlate with findings noted in other topical ASM 981 toxicity studies conducted in CD-1 mice. The 100 mg/kg/day dose was associated with lymphoproliferative changes indicative of immunossuppression. The thymic atrophy noted after 4, 9 or 13 weeks of treatment was reversible. Pleomorphic lymphoid proliferation was first noted after 9 weeks of treatment and was also present in the recovery group for the 13 week duration of treatment group.

Repeat Dose Toxicology Study #9:

Oncogenicity study by dermal administration to CD-1 mice for 52 weeks

Study Title:	Oncogenicity study by dermal administration to CD-1 mice for 52 weeks
Study No:	T-92/203-182
Contract Study No:	SPM102/970488
Volume #, and page #:	58, 5-1

Conducting laboratory: _____
Date of study initiation: 4/23/96
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 50 µl 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 (except untreated controls) were dosed once each day, 7 days/week, for a duration of either 34 weeks or 52 weeks.

It is important to note that the initial objective of this study was to assess the oncogenic potential of ASM 981 after topical treatment. Apparently this was objective was changed by the sponsor during week 34 of treatment. The new objectives became to investigate effects of ASM 981 on thymus and other lymphoid tissues and to confirm that the high dose would be free of pleomorphic lymphoproliferative changes after 52 weeks. Therefore, the sponsor requested an interim analysis at 34 weeks and then a final analysis at 52 weeks.

Dosing:

- *species/strain:* CD-1 mice
- *#/sex/group or time point:* 60/sex/dose (Note: 20/sex/dose were sacrificed after 34 weeks of treatment and the remainder after 52 weeks)
- *satellite groups used for toxicokinetics or recovery:* 24/sex/dose for TK analysis
- *age:* 35 - 42 days
- *weight:* males: 25 - 38 g; females: 20 - 30 g
- *doses in administered units:* 0 (untreated control), 0 (ethanol control), 0.5, 1.5 and 5.0 mg/kg/day for males and
  0 (untreated control), 0 (ethanol control), 0.6, 2.0 and 6.6 mg/kg/day in females
- *route, form, volume, and infusion rate:* topical; ethanol solution; 50 µl/mouse of 0.4, 1.2 or 4.0 mg/ml ASM 981

Observations and times:

- *Clinical signs:* daily
- *Body weights:* weekly
- *Food consumption:* weekly
- *Gross pathology:* at necropsy at week 34 or 52 of treatment
- *Histopathology:* The following organs were preserved for histological analysis from all treated animals (for weeks 34 and 52): lymph nodes

(axillary, mandibular, mesenteric, trancheobronchial), spleen and thymus.

- *Toxicokinetics:* Blood samples were obtained from animals (3/sex/dose) at 1, 2, 4, 7 and 24 hours after dosing during weeks 4, 34 or 52 of treatment. Mesenteric lymph node samples for TK analysis were obtained from main study and TK animals after 34 or 52 weeks of treatment. ASM 981 levels were determined in the blood and tissue samples by ———— ———— The limit of quantification was — ng/ml for blood and — mg/g for pooled mesenteric lymph nodes.

Results:

- **Clinical signs** No treatment related effects on clinical signs or 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.
- **Gross pathology** No treatment related effects on gross pathology parameters were noted in this study.
- **Histopathology** Microscopic examination of thymus, spleen and selected lymph nodes did not reveal thymus medullary atrophy, pleomorphic proliferation, lymphomas in the lymphatic tissues or other neoplastic findings that were clearly attributed to treatment. Follicular cell lymphomas and lymphoblastic lymphomas that were noted after 34 and 52 weeks of treatment showing a similar distribution pattern for control and treated groups. After 34 weeks of treatment, slightly higher incidences of thymus medullary hyperplasia and hyperplasia of the white pulp in the spleen were noted predominantly for males in the mid and high dose groups. An increased incidence of white pulp hyperplasia in the spleen was noted in treated females. These findings were no longer observed in animals that were sacrificed after 52 weeks of treatment.
- **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_{0-24hr} (ng•hr/ml)		
		Week 4	Week 32	Week 54	Week 4	Week 32	Week 54	Week 4	Week 32	Week 54
0.5	Male	18.3	13.3	10.1	5	5	7	302	225	182
0.6	Female	18.0	22.3	23.4	1	7	5	268	395	355
1.5	Male	32.8	17.2	17.4	5	5	5	364	225	251
2.0	Female	22.8	19.0	25.2	7	1	5	446	333	456
5	Male	38.6	26.3	27.7	1	2	2	488	334	424
6.6	Female	42.9	40.5	59.3	7	2	7	717	646	833

A dose dependent increase in systemic exposure was noted in this study. Female satellite study mice appeared to have higher systemic exposure during the 52 week treatment period. This was probably due to slightly higher doses of ASM 981 being administered topically due to lower body weight compared to male mice. No indication of drug accumulation in the blood was noted in this study.

The mesenteric lymph node concentrations of ASM 981 (mean) are presented in the following table.

Dose (mg/kg/day)	Sex	Lymph Node Concentration (ng/g)	
		Week 34	Week 54
0.5	Male	13.4	11.4
0.6	Female	15.3	18.0
1.5	Male	23.1	20.7
2.0	Female	19.4	17.5
5	Male	40.3	42.2
6.6	Female	43.3	65.5

A dose dependent increase in ASM 981 concentration in the lymph nodes was noted in this study. The ratios of ASM 981 levels in lymph nodes to blood for both genders were between 1.6 and 3.1 for the low dose, between 1.8 and 4.2 for the mid dose and between 4.0 and 6.7 for the high dose. These ratios suggest that the compound distributed into the lymph nodes in preference to the blood as the dose was increased and may partially contribute to the lymph node being a target organ for ASM 981. No indication of drug accumulation in the lymph nodes was noted in this study.

Summary of individual study findings:

Daily topical treatment with an ethanolic solution of ASM 981 (0.5, 1.5 and 5.0 mg/kg/day for males and 0.6, 2.0 and 6.6 mg/kg/day for females) for 52 weeks did not cause any severe adverse effects on the skin. No skin papillomas or other neoplastic masses were observed in the skin in this study. A slight increase in the incidence of medullary hyperplasia in the thymus and hyperplasia of the white pulp was observed in the spleens of males in the mid and high dose groups after 34 weeks of treatment. These changes were transient in nature since they were not observed after 52 weeks of treatment and are probably secondary to the pharmacological action of ASM 981.

The sponsor had terminated the current study early due to the results noted in 13 week oral and dermal toxicity studies conducted in mice. The 13 week dermal toxicity study was conducted with the ethanolic solution of ASM 981. The doses used in the both the oral and dermal 13 week studies were higher than those used in this 52 week study.

The results of the 13 week oral toxicity study conducted in CD-1 mice identified potential target organs of toxicity as the pancreas, thymus, spleen/mesenteric lymph nodes and uterus/vagina. The effects noted in the thymus and spleen/mesenteric lymph nodes were probably related to the pharmacological (immunosuppressive) activity of ASM 981. The NOAEL identified in this study was 10 mg/kg/day ($AUC_{0.5\text{-}4hr}$ = 1029 and 2949 ng·hg/ml in males and females, respectively) for mice after 13 weeks of oral administration of ASM 981.

The focus of one of the 13 week dermal toxicity studies in CD-1 mice was to assess the dose response relationship of immunousuppression and lymphoproliferative disorders following dermal administration of ASM 981 (ethanolic solution). 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 ($AUC_{0\text{-}24hr}$ = 643 and 675 ng·hr/ml for males and females, respectively) for mice after 13 weeks of topical administration of ASM 981 dissolved in ethanol. Lymphoproliferative changes were noted at 25 mg/kg/day ($AUC_{0\text{-}24hr}$ = 1854 and 1745 ng·hr/ml for males and females, respectively) after 13 weeks of topical administration of the ethanolic ASM 981 solution.

The highest dose tested in the 52 week study was less than the NOAEL dose in the 13 week oral toxicity study in CD-1 mice and less than the dose that demonstrated lymphoproliferative changes in the 13 week dermal toxicity study in CD-1 mice. The highest dose tested in this 52 week dermal toxicity study was 5.0 mg/kg/day for males (AUC = 424 ng·hr/ml after 52 weeks) and 6.6 mg/kg/day in females (AUC = 833 ng·hr/ml after 52 weeks). It is not terribly surprising that no significant toxicity effects were noted in the 52 week study. If this study had continued, the review of this study would have determined that an adequate dose selection was not used for this study. The sponsor did repeat the mouse dermal carcinogenicity study with an ethanolic ASM 981 solution. This study is reviewed later in this document. Typically the dermal carcinogenicity study for a particular drug product is conducted with the final to be marketed formulation. Therefore, the sponsor was informed that a dermal carcinogenicity study with the final marketed formulation for the ASM 981 cream formulation is recommended. The sponsor conducted a dermal carcinogenicity study in the rat with the final marketed formulation of the ASM 981 cream, which is reviewed later in this document.

Oral Rat:

Repeat Dose Toxicology Study #10:

4-week oral reproductive hormone study in male rats

Note: The purpose of this oral toxicity study in male rats was to investigate a histopathological indication of reproductive hormone suppression and its possible relationship to thymoma formation noted in the oral rat carcinogenicity study.

Study Title:	4-week oral reproductive hormone study in male rats
Study No:	T-95/BS-728
Novartis Study No:	001064
Volume #, and page #:	63, 5-1
Conducting laboratory:	Novartis Pharma AG, Basel, Switzerland
Date of study initiation:	5/4/2000
GLP compliance:	Yes
QA- Report:	Yes (X) No ()
Drug, and lot#:	ASM 981 – batch# X276 1095
Formulation/vehicle:	Aqueous solution containing 0.5% Poloxamer 188 and 3.5% hydroxypropylmethyl cellulose (Note: The stock oral ASM 981 solution was referred to as the solid dispersion and consisted of 20% ASM 981 (w/w) solid dispersion in 0.5% Poloxamer 188 and 3.5% hydoxypropylmethyl cellulose as a carrier in water.)

Methods:

Test article or vehicle (aqueous solution containing 0.5% Poloxamer 188 and 3.5% hydroxypropylmethyl cellulose) was administered orally (via gavage) on a daily basis, 7 days/week, for 4 weeks.

Dosing:

- *species/strain:* Male Wistar rats
- *#/sex/group or time point:* 15 males/dose
- *satellite groups used for toxicokinetics or recovery:* NA
- *age:* 13 - 18 weeks
- *weight:* males: 241 - 299 g
- *doses in administered units:* 0, 10 and 40 mg/kg ASM 981
- *route, form, volume, and infusion rate:* oral; liquid dispersion; 5 ml/kg/day

Observations and times:

- *Clinical signs:* daily
- *Body weights:* weekly
- *Food Consumption:* weekly
- *Reproductive Hormone*

Determination: pretest and at end of study (Testosterone, Luteinising hormone and Follicle stimulating hormone levels were measured)

- *Gross pathology:* at sacrifice
- *Organ weights:* Brain, epididymides, pituitary, prostate, seminal vesicles, testes and thymus
- *Histopathology:* The following organs were preserved in 10% buffered formalin from all animals: Brain, epididymides, pituitary, prostate, seminal vesicles, testes and thymus and all gross lesions.

  All listed organs were examined microscopically from all treated animals.

- *Toxicokinetics:* A blood sample was collected from each animal towards the end of the last treatment week at approximately 4 pm. These blood samples were used to determine the concentration of ASM 981 in whole blood by ———————

Results:

- **Clinical signs** No treatment related effects on mortality or clinical signs were noted in this study.

- **Body weights** Decreased body weight was noted in high dose animals (↓8.9%).

- **Food Consumption** No treatment related effects on food consumption were noted in this study.

- **Reproductive Hormone Determination** A decrease in median serum testosterone levels was noted in low (↓65%) and high dose animals (↓35%) compared to control animals. This effect was not dose dependent. The study report states that a high inter-individual variability of serum testosterone levels makes the comparison of data difficult. The study report states that the variability can be explained by the pulsatile release of testosterone and the influence that external factors such as stress can have on testosterone levels.

  No changes in lutenising hormone or follicle stimulating hormone levels were noted in this study.

- **Gross pathology** No treatment related gross pathology effects were noted in this study.

- **Organ Weights** A significant decrease in prostrate weight was noted in low (↓19.4%) and high dose animals (↓28.6%). A significant decrease in pituitary weight was noted in low (↓20.0%) and high dose animals (↓22.2%).

A significant decrease in thymus weight was noted in low (↓9.1%) and high dose animals (↓13.6%).

- **Histopathology** — Treatment related findings were noted in the thymus. A dose dependent increase in severity and the incidence of medullary atrophy (minimal to moderate) and cortical lymphocytolysis (minimal to slight) was noted in low and high dose animals.

  The study report states that no treatment related effects on the quality of the different stages of spermatogenesis or findings indicative of a hormonal disturbance in the pituitary were noted in this study.

- **Toxicokinetics** — Mean ± SD ASM 981 concentrations were 32.7 ± 11.8 ng/ml and 103 ± 24 ng/ml for low and high dose animals, respectively.

Summary of individual study findings:

The decrease in thymus weights and the thymic findings of medullary atrophy and cortical lymphocytolysis at both dose levels are consistent with findings noted in other ASM 981 toxicity studies. These changes are indicative of systemic immunosuppression related to the pharmacological action of ASM 981. The results from this study also suggest that moderate suppression of testosterone secretion may be a toxic effect associated with ASM 981 administration. This effect was not dose dependent and no related morphological changes were noted for this effect. It is unclear how a moderate suppression of testosterone secretion could have a possible relation to the thymoma noted in the oral rat carcinogenicity study. The thymoma noted in the oral rat carcinogenicity study is probably more related in systemic immunosuppression associated with ASM 981 administration.

Repeat Dose Toxicology Study #11:

4-week oral reproductive hormone study in female rats

Note: The purpose of this oral toxicity study in female rats was to investigate a histopathological indication of reproductive hormone suppression and its possible relationship to thymoma formation noted in the oral rat carcinogenicity study.

Study Title:	4-week oral reproductive hormone study in female rats
Study No:	T-96/BS-730
Novartis Study No:	001065
Volume #, and page #:	63, 5-149
Conducting laboratory:	Novartis Pharma AG, Basel, Switzerland
Date of study initiation:	5/4/2000
GLP compliance:	Yes
QA- Report:	Yes (X) No ()
Drug, and lot#:	ASM 981 – batch# X276 1095

Formulation/vehicle: Aqueous solution containing 0.5% Poloxamer 188 and 3.5% hydroxypropylmethyl cellulose (Note: The stock oral ASM 981 solution was referred to as the solid dispersion and consisted of 20% ASM 981 (w/w) solid dispersion in 0.5% Poloxamer 188 and 3.5% hydoxypropylmethyl cellulose as a carrier in water.)

Methods:

Test article or vehicle (aqueous solution containing 0.5% Poloxamer 188 and 3.5% hydroxypropylmethyl cellulose) was administered orally (via gavage) on a daily basis, 7 days/week, for 4 weeks.

Dosing:

- *species/strain:* Female Wistar rats
- *#/sex/group or time point:* 30 females/dose
- *satellite groups used for toxicokinetics or recovery:* TK analysis - 5 females/dose
- *age:* 15 - 21 weeks
- *weight:* males: 199 - 263 g
- *doses in administered units:* 0, 10 and 40 mg/kg ASM 981
- *route, form, volume, and infusion rate:* oral; liquid dispersion; 5 ml/kg/day

Observations and times:

- *Clinical signs:* daily
- *Body weights:* weekly
- *Food Consumption:* weekly
- *Vaginal Cytology:* daily; vaginal saline washes were fixed, stained and examined by a pathologist; used to determine the stage of estrous cycle and to determine if each estrous cycle was normal or not
- *Reproductive Hormone Determination:* pretest and at end of study; Estrogen (10 animals/dose), progesterone (10 animals/dose), Luteinising hormone and Follicle stimulating hormone levels (10 animals/dose for LH and FSH); hormone levels were synchronized with respect to the stage of the estrous cycle
- *Gross pathology:* at sacrifice
- *Organ weights:* Brain, ovaries, pituitary, thymus and uterus
- *Histopathology:* The following organs were preserved in 10% buffered formalin from all animals: Brain, cervix, mammary gland, ovaries, pituitary, thymus, uterus, vagina and all gross lesions.

  All listed organs were examined microscopically from all treated animals.

- *Toxicokinetics:* A blood sample was collected from each satellite TK animal once daily for 4 consecutive days during the last week of

treatment. Blood samples were obtained at approximately 4 pm each day. The first blood sample was generally taken on the first day of the new estrous cycle. These blood samples were used to determine the concentration of ASM 981 in whole blood by ———————

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.

- **Vaginal Cytology** — A dose related increase in the estrous cycle length was noted in this study. Transiently prolonged (6-10 days without estrous) or acyclic (over 10 days without estrous) phases were seen during the treatment period which were always followed by a normal cyclic activity. Normal estrous cycle in the female Wistar rat is 3 – 5 days. The prolonged phases were seen throughout the treatment period. In addition, permanently prolonged or acyclic phases were observed which lasted until the end of the treatment phase and were not followed by normal cyclic activity. In most of these animals, the vaginal smears showed a high cellularity and the presence of mucous cells, uncharacteristic of any normal stage of the reproductive cycle.

- **Reproductive Hormone Determination** — Pretest measurements demonstrated that estrogen serum levels were lowest in estrus and metestrous, rose during diestrus and reached a peak during proestrus. High dose animals showed a significant decrease in estrogen levels during diestrus (↓81.8%) and proestrus (↓68.4%) compared to their respective pretest values. Estrogen could not be detected in four high dose animals.

  Pretest measurements demonstrated that progesterone levels reached a peak a metestrus. The study report states that only the onset of the second pear at proestrus could be shown with the data from this study (due to the time of day at which the blood samples were collected). A significant decrease in progesterone level was noted during proestrus in high dose animals (↓46.4%).

  No significant differences between basal lutenising hormone or follicle stimulating hormone levels were noted in this study.

- **Gross pathology** A small uterus and ovaries were noted in one high dose animal.

- **Organ Weights** A significant decrease in pituitary weight was noted in low (↓15.1%) and high dose animals (↓25.4%).

- **Histopathology** Treatment related findings were noted in the thymus, ovaries, uterus and vagina.

Thymus:

A dose dependent increase in severity of slight to massive medullary atrophy and/or minimal to slightly increased incidence of cortical lymphocytolysis was noted in all low and high dose animals. Slight focal lymphoid hyperplasia was observed in one high dose animal.

Ovaries:

A dose related increased incidence of slight atrophy, consisting of inactive intestinial glands (characterized by cytoplasmic rarefaction) and/or reduced number of corpora lutea was noted in individual low and high dose animals.

Uterus:

A dose related increased incidence and severity of minimal to moderate atrophy was noted in individual low and high dose animals.

Vagina:

A dose related increased incidence of moderate epithelial atrophy and mucification was noted in individual low and high dose animals. This finding was consistent with an acyclic stage of the reproductive cycle.

- **Toxicokinetics** The ASM 981 blood concentrations (mean ± SD) 1 – 4 days after the start of the estrus cycle are provided in the following table.

Dose (mg/kg)	Days after start of estrous cycle	ASM 981 concentration (ng/ml)
10	1	31.7 ± 14.6
	2	46.7 ± 31.8
	3	44.5 ± 25.7
	4	53.4 ± 38.7
40	1	484 ± 168
	2	378 ± 177
	3	967 ± 524
	4	755 ± 217

In general, the ASM 981 concentrations at the start of the estrous cycle were lower than those found on the following days.

Summary of individual study findings:

The decrease in thymus weights and the thymic findings of medullary atrophy and cortical lymphocytolysis at both dose levels are consistent with findings noted in other ASM 981 toxicity studies. These changes are indicative of systemic immunosuppression related to the pharmacological action of ASM 981.

Estrogen levels were decreased in high dose animals during diestrus and proestrus. This hormonal disruption was reflected in the decreased pituitary weights, alteration of the estrous cycle length and atrophic changes noted in the ovary, uterus and vagina. The significant decrease in progesterone levels noted in high dose animals could be interpreted to correspond with the observed decreases in estrogen levels and the histopathological findings. However, this correlation should be evaluated with more caution than the correlation with estrogen because blood sampling time only detected the onset of the peak of progesterone. The study report states that blood sampling obtained 2 – 3 hours later in the day would have been more beneficial for progesterone level analysis.

According to the study report, the morphological alterations in the reproductive organs were consistent with Type I reproductive toxicity described in the literature as being an effect on the central nervous system that may disturb the regulation of gonadotropin section. The changes may be a result of effects on the hypothalamic-pituitary-ovarian-endometrial axis. The sponsor proposes that it is possible that this effect may also play a factor in the thymoma formation noted in the oral carcinogenicity study in Wistar rats. It is unclear how this may be a possible factor in thymoma formation. It is more probable that the noted thymoma is probably related to the immunosuppressive properties associated with ASM 981.

Repeat Dose Toxicology Study #12:

A 13-week oral (per gavage) toxicity study in rats (Drug form: Lyophilisate suspension)

Study Title:	A 13-week oral (per gavage) toxicity study in rats (Drug form: Lyophilisate suspension)
Study No:	T-26/203-095
Sandoz Study No:	435R
Volume #, and page #:	25, 5-1
Conducting laboratory:	Sandoz Pharma LTD, Basel, Switzerland
Date of study initiation:	11/30/94
GLP compliance:	Yes
QA- Report:	Yes (X) No ()
Drug, and lot#:	ASM 981 – batch# Y292 1094
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:* Wistar rats
- *#/sex/group or time point:* 10/sex/dose
- *satellite groups used for toxicokinetics or recovery:* 4/sex/dose for TK analysis; 6/sex/dose in vehicle and high dose groups in 4 week recovery group
- *age:* 8 weeks
- *weight:* males: 201 - 250 g; females: 134 - 177 g
- *doses in administered units:* 0, 2, 10 and 50 mg/kg ASM 981
- *route, form, volume, and infusion rate:* oral; liquid dispersion; 5, 0.2, 1 and 5 ml/kg/day for vehicle, low dose, mid dose and high dose groups, respectively.

Observations and times:

- *Clinical signs:*	daily
- *Body weights:*	weekly
- *Food Consumption:*	weekly
- *Ophthalmology:*	pretest, day 84 (main study animals) and day 111 (recovery animals)
- *Hematology:*	pretest, day 92 (main study animals) and day 114 (recovery animals)
- *Clinical chemistry:*	pretest, day 92 (main study animals) and day 114 (recovery animals)
- *Urinalysis:*	pretest, day 92 (main study animals) and day 114 (recovery animals)

- *Gross pathology:* at sacrifice
- *Organ weights:* Adrenals, brain, heart, kidneys, liver, pancreas, pituitary gland, prostrate/uterus, spleen, testes/ovaries, thymus, thyroid glands
- *Histopathology:* The following organs were preserved in 10% buffered formalin from all animals: adrenals, aorta, bones (femur/knee/tibia, right), brain, esophagus, eyes, 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 listed organs were examined microscopically from the control and high dose group animals. In addition, pancreas, stomach, kidneys, thymus, spleen, mesenteric and mandibular lymph nodes, lungs, prostrate, seminal vesicles, ovaries, uterus, vagina, thyroids, brain, spinal cord and sciatic nerve from animals in the low and mid dose groups and recovery groups were examined microscopically.

- *Toxicokinetics:* Blood samples were obtained from animals (4/sex/dose) at 0.5, 1, 2, 4, 7 and 24 hours after the first (day 1) and last dose (day 92). ASM 981 levels were determined in the blood samples by [redacted] The limit of quantification was — ng/ml.

Results:

- **Clinical signs** No treatment related effects on mortality or clinical signs were noted in this study.

- **Body weights** A decrease in overall body weight was noted in high dose animals (males: ↓9.3%; females: ↓5.4%) compared to control animals. Body weight returned to normal by the end of the recovery period in high dose animals.

- **Food Consumption** No treatment related effects on food consumption were noted in this study.

- **Ophthalmology** Lenticular changes (optical discontinuity between cortex and nucleus that are consistent with cataract development) were noted in 3/10 high dose males.

- **Hematology** Lymphocyte counts were reduced in mid dose females (↓32.0%) and high dose animals (males: ↓23.7%; females: ↓27.5%) compared to control animals. Basophil counts were reduced in high dose (males: ↓47.4%; females: ↓54.8%) animals compared to control animals. Eosinophil counts were increased in mid (↑1.4X) and high dose males (↑1.3X) compared to control males.

  The effected hematological parameters returned to normal in high dose recovery animals.

- **Clinical Chemistry** A decrease in glucose levels was noted in high dose animals (males: ↓27.3%; females: ↓28.7%) compared to control animals. A dose dependent decrease in magnesium levels was noted in mid (males: ↓8.6%; females: ↓11.8%) and high dose animals (males: ↓31.7%; females: ↓25.4%) compared to control animals. Blood urea nitrogen was increased in high dose animals (males: ↑1.3X; females: 1.5X) compared to control animals. Triglyceride levels were increased in high dose females (↑1.5X) compared to control females. Cholesterol levels were increased in high dose males (↑1.3X) compared to control males.

  The effected clinical chemistry parameters returned to normal in high dose recovery animals.

- **Urinalysis** Urinary volume was increased in high dose animals (males: ↑2.0X; females: ↑2.7X) compared to control animals. Urinary volume returned to normal in high dose recovery animals.

- **Gross pathology** No treatment related gross pathology effects were noted in this study.

- **Organ Weights** A significant decrease in prostrate weight was noted in high dose males (↓38.6%). A significant decrease in pituitary weight was noted in high dose females (↓31.6%).

- **Histopathology** Treatment related findings were noted in the thymus, kidneys, lungs, pancreas, glandular stomach, sternum, spleen and mesenteric and mandibular lymph nodes.

Thymus:

Medullary atrophy was present in mid (males: 9/10; females: 5/10) and high dose animals (males: 10/10; females: 10/10). The incidence of lymphophagocytosis was equivalent for all groups (10/10). However, the severity of this lesion increased with increased dose for the mid and high dose groups. No treatment related lesions were noted in recovery animals.

Kidneys:

The incidence/severity of tubular basophilia was increased in high dose animals (males: 10/10; females: 10/10) compared to control animals (males: 2/10; females: 4/10). The incidence/severity of tubular corticomedullary mineralization was increase in high dose males (10/10) compared to control males (1/10). The incidence of tubular corticomedullary mineralization was equivalent in all female groups (10/10). However the severity of this lesion was increased in high dose females compared to control females. A slight increase in the severity of tubular corticomedullary mineralization was still noted in high dose recovery animals.

Lungs:

The severity of perivascular/peribronchiolar inflammatory cells was increased in high dose animals compared to control animals. This effect was not noted in recovery animals.

Pancreas:

Minimal to moderate islet cell vacuolation was present in high dose animals (males: 10/10; females: 5/10). No treatment related lesions were noted in recovery animals.

Glandular stomach:

Minimal to moderate submucosal edema was noted in high dose animals (males: 2/10; females: 2/10). A slight increase in the severity of submucosal inflammatory infiltrates was noted in high dose animals. No treatment related lesions were noted in recovery animals.

Sternum:

Minimal to mild bone marrow depletion was noted in high dose animals (males: 6/10; females: 6/10). This effect was still present in high dose recovery animals (males: 3/6; females: 2/6).

Mesenteric and mandibular lymph nodes:

Reduced germinal center formation in mesenteric lymph nodes was noted in high dose animals (males: 1/10; females: 3/10) compared to control animals (males: 7/10; females: 6/10). Reduced germinal center formation in mandibular lymph nodes was noted in high dose animals (males: 3/10; females: 7/10) compared to control animals (males: 7/10; females: 8/10). No treatment related effects were noted in recovery animals.

Spleen:

Extramedullary hematopoiesis was noted with the same incidence for all groups (10/10). However, the extent of extramedullary hematopoiesis was reduced in high dose animals compared to control animals. No treatment related effects were noted in recovery animals.

- **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)		$AUC_{0-24\ hr}$ (ng·hg/ml)	
		Day 1	Day 92	Day 1	Day 92	Day 1	Day 92
2	Male	21	9	0.5	0.5	--	--
	Female	8	9	1	0.5	--	--
10	Male	321	355	1	1	766	605
	Female	56	57	0.5	0.5	344	203
50	Male	944	1118	1	2	3481	5977
	Female	454	587	2	0.5	3727	2398

A dose dependent increase in systemic exposure was noted in this study. It is interesting to note that no AUC level could be calculated for the low dose group in this study. No indication of drug accumulation was noted in this study (except for the high dose male group). Males tended to have higher C_{max} values compared to females. Males may have a slightly higher systemic exposure compared to females.

Summary of individual study findings:

Potential target organs of toxicity identified in this study included the thymus, kidneys, lungs, pancreas, glandular stomach, bone marrow (sternum), spleen and mesenteric and mandibular lymph nodes. In addition, eyes were identified as a possible target organ due to noted lenticular changes in high dose males. The prostrate may be a potential target organ due to the decrease in organ weight even though there was no corresponding histopathological effects. The effects noted in the thymus, spleen and mesenteric and mandibular lymph nodes are probably related to the pharmacological (immunosuppressive) activity of ASM 981. The effects noted in all of the other potential target organs were probably related to overt toxicity associated with ASM 981. The NOAEL identified in this study was 2 mg/kg/day (no AUC values could be determined for this dose) for rats after 13 weeks of oral administration of ASM 981 (lyophilisate suspension).

Repeat Dose Toxicology Study #13:

A 26-week oral (per gavage) toxicity study in rats (Drug form: solid dispersion)

Study Title: A 26-week oral (per gavage) toxicity study in rats (Drug form: solid dispersion)
Study No: T-27/203-165
Sandoz Study No: 444R

Volume #, and page #:	27, 5-1
Conducting laboratory:	Sandoz Pharma LTD, Basel, Switzerland
Date of study initiation:	11/20/95
GLP compliance:	Yes
QA- Report:	Yes (X) No ()
Drug, and lot#:	ASM 981 – batch# X148 0695
Formulation/vehicle:	Aqueous solution containing 0.5% Poloxamer 188 and 3.5% hydroxypropylmethyl cellulose (Note: The stock oral ASM 981 solution was referred to as the solid dispersion and consisted of 20% ASM 981 (w/w) solid dispersion in 0.5% Poloxamer 188 and 3.5% hydoxypropylmethyl cellulose as a carrier in water.)

Methods:

Test article or vehicle (aqueous solution containing 0.5% Poloxamer 188 and 3.5% hydroxypropylmethyl cellulose) was administered orally (via gavage) on a daily basis, 7 days/week, for 26 weeks.

Dosing:

- *species/strain:* Wistar rats
- *#/sex/group or time point:* 20/sex/dose
- *satellite groups used for toxicokinetics or recovery:* 8/sex/dose for TK analysis; 6/sex/dose in vehicle and high dose groups in 7 week recovery group
- *age:* 8 weeks
- *weight:* males: 192 - 251 g; females: 131 - 177 g
- *doses in administered units:* 0, 1, 5 and 25 mg/kg ASM 981
- *route, form, volume, and infusion rate:* oral; solid dispersion; 5 ml/kg/day

Observations and times:

- *Clinical signs:*	daily
- *Body weights:*	weekly
- *Food Consumption:*	weekly
- *Ophthalmology:*	pretest, week 25 (main study animals) and week 30 (recovery animals)
- *Hematology:*	pretest, week 26 (main study animals) and week 33 (recovery animals)
- *Clinical chemistry:*	pretest, week 26 (main study animals) and week 33 (recovery animals)
- *Urinalysis:*	pretest, week 26 (main study animals) and week 33 (recovery animals)
- *Gross pathology:*	at sacrifice
- *Organ weights:*	Adrenals, brain, heart, kidneys, liver, pancreas, pituitary gland, prostrate/uterus, spleen, testes/ovaries, thymus, thyroid glands
- *Histopathology:*	The following organs were preserved in 10% buffered formalin from all animals: adrenals, aorta, bones (femur/knee/tibia, right), brain, esophagus, eyes, 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 listed organs were examined microscopically from the control and high dose group animals. In addition, brain, spinal cord, sciatic nerve, thymus, tracheobronchial, mandibular and mesenteric lymph nodes, spleen, lungs, bone (joint femur/tibia), sternum, pancreas, eyes, kidneys, urinary bladder, thyroids, prostate and salivary glands from animals in the low and mid dose groups and recovery groups were examined microscopically.

- *Toxicokinetics:* Blood samples were obtained from animals (4/sex/dose) at 0.5, 1, 2, 4, 7 and 24 hours after dosing during week 5, 14 and 27. ASM 981 levels were determined in the blood samples by [redacted] The limit of quantification was [redacted] ng/ml.

Results:

- **Clinical signs** No treatment related effects on mortality or clinical signs were noted in this study.

- **Body weights** A moderate decrease in overall body weight was noted in high dose males (↓15.4%) compared to control males. A moderate decrease in overall body weight was still apparent in high dose recovery males (↓15.5%) compared to control males. In addition, a slight decreased in overall body weight was noted in high dose recovery females (↓5.8%) compared to control females.

- **Food Consumption** No treatment related effects on food consumption were noted in this study.

- **Ophthalmology** Lenticular changes (optical discontinuity between lens cortex and nucleus) that are consistent with cataract development were noted in high dose animals (males: 16/18; females: 17/19). The same lenticular changes were noted in high dose recovery animals (males: 6/6; females: 5/6). No evidence of recovery or regression from the lenticular findings was apparent in this study.

- **Hematology** — No treatment related effects on hematological parameters were noted in this study.

- **Clinical Chemistry** — A decrease in glucose levels was noted in high dose females (↓33.3%) compared to control females. A dose dependent decrease in magnesium levels was noted in mid (males: ↓7.7%; females: ↓8.8%) and high dose animals (males: ↓32.7%; females: ↓28.9%) compared to control animals. Blood urea nitrogen was increased in high dose animals (males: ↑1.5X; females: 1.7X) compared to control animals. Triglyceride levels were increased in high dose females (↑1.7X) compared to control females. Cholesterol levels were increased in high dose males (↑1.3X) compared to control males.

  The affected clinical chemistry parameters returned to normal in high dose recovery animals.

- **Urinalysis** — Urinary volume was increased in high dose animals (males: ↑2.1X; females: ↑2.3X) compared to control animals. Sodium levels were increased in high dose animals (males: ↑1.7X; females: ↑1.5X) compared to control animals. Calcium levels were increased in high dose animals (males: ↑6.1X; females: ↑1.4X) compared to control animals. Chloride levels were increased in high dose animals (males: ↑2.5X; females: ↑2.1X) compared to control animals.

  The effected urinary parameters returned to normal in high dose recovery animals.

- **Gross pathology** — A small prostate was noted in 7 high dose males. A granulated surface of the corticomedullary junction or of the whole kidney, and/or pale discoloration of the corticomedullary junction was noted in several high dose males and females.

- **Organ Weights** — A significant decrease in prostrate weight was noted in high dose males (↓55.6%). A significant decrease in pituitary weight was noted in high dose females (↓36.8%). A decrease in pancreas weight was noted in high dose females (↓18.5%). A decrease in spleen weight was noted in high dose females (↓16.4%). A decrease in adrenal gland weight was noted in high dose females (↓16.0%).

- **Histopathology** — Treatment related findings were noted in the thymus, kidneys, lungs, pancreas, sternum, spleen, mesenteric and mandibular lymph nodes, prostate, salivary glands, urinary bladder and eyes.

Thymus:

Medullary atrophy was present in mid (males: 19/20; females: 14/20) and high dose animals (males: 20/20; females: 20/20). Multifocal to diffuse, slight to moderate cortical hyperplasia was present in mid (males: 3/20; females: 6/20) and high dose animals (males: 4/20; females: 7/20). Lymphophagocytosis in the cortex was noted in high dose animals (males: 8/20; females: 9/20).

Malignant lymphoma was noted in one high dose recovery male. No other treatment related lesions were noted in recovery animals.

Kidneys:

The incidence/severity of tubular basophilia was increased in high dose animals (males: 20/20; females: 20/20) compared to control animals (males: 3/20; females: 6/20). The incidence/severity of tubular corticomedullary mineralization was increase in high dose animals (males: 20/20; females: 20/20) compared to control animals (males: 1/20; females: 19/20). For high dose females the increase in severity of tubular corticomedullary mineralization was the most notable feature. Tubular vacuolation was present in high dose animals (males: 13/20; females: 8/20). The incidence/severity of tubular atrophy associated with interstitial lymphocytic leukocytes was increased in high dose animals (males: 16/20; females: 20/20) compared to control animals (males: 0/20; females: 5/20). An increased presence of globule leukocytes in the pelvic urothelium was noted in high dose animals (males: 16/20; females: 13/20) compared to control animals (males: 2/20; females: 5/20).

An increase in the incidence/severity of tubular corticomedullary mineralization was still noted in high dose recovery animals (males: 5/6; females: 6/6) compared to control recovery animals (males: 0/6; females: 5/6). Once again the increase in severity of tubular corticomedullary mineralization in high dose recovery females was the most notable feature even though the incidence rate was approximately equivalent to control recovery animals. An increase in tubular atrophy associated with interstitial lymphocytic leukocytes was still noted in high dose recovery animals (males: 6/6; females: 6/6) compared to control recovery animals (males: 0/6; females: 0/6).

Lungs:

Minimal to slight chronic peribronchitis/peribronchiolitis was noted in high dose animals (males: 3/20; females: 7/20). Traces of peribronchiolar pigment was noted in high dose animals (males: 1/20; females 5/20). Minimal increase in pervascular infiltrates was noted in high dose animals (males: 4/20; females: 3/20)

Minimal chronic peribronchitis/peribronchiolitis was noted in 1/6 high dose recovery females. Traces of peribronchilar pigment was noted in 2/6 high dose recovery females.

Pancreas:

Minimal to slight islet cell vacuolation was present in high dose animals (males: 15/20; females: 17/20). Minimal/slight to marked islet cell depletion was noted in high dose animals (males: 20/20; females: 20/20). Slight to marked islet cell depletion was still noted in high dose recovery animals (males: 6/6; females: 5/6). The study report states that immunohistochemisty demonstrated that the insulin secreting cell population of the islet was affected.

Sternum:

Minimal to mild bone marrow depletion was noted in high dose animals (males: 12/20; females: 16/20). Bone marrow atrophy was noted in high dose animals (males: 14/20; females; 17/20). This effects was still present in high dose recovery animals (bone marrow depletion – males: 6/6; females: 4/6 and bone marrow atrophy – males: 1/6; females: 3/6).

Mesenteric and mandibular lymph nodes:

Reduced germinal center formation in mandibular lymph nodes was noted in high dose animals (males: 8/20; females: 2/20) compared to control animals (males: 13/20; females: 10/20). Lymphoid depletion in mesenteric lymph nodes was noted in mid (males: 10/20; females: 7/20) and high dose animals (males: 20/20; females: 15/20). Lymphoid depletion in mandibular lymph nodes was noted in high dose animals (males: 4/20; females: 7/20). No treatment related effects were noted in recovery animals.

Spleen:

Extramedullary hematopoiesis was noted with the same incidence for all groups (20/20). However, the extent of extramedullary hematopoiesis was reduced in high dose animals compared to control animals. No treatment related effects were noted in recovery animals.

Prostate:

Slight to moderate atrophy was noted in 10/20 high dose males. Slight atrophy was present in 1/6 high dose recovery males.

Salivary glands:

Diffuse, minimal to slight atrophy in the glandula submandibularis was noted in 11/20 high dose males and in the glandula parotis in 2/20 high dose males. Minimal atrophy in the glandula submandibularis was noted in 2/6 high dose recovery males.

Urinary Bladder:

Minimal to slight urothelial hyperplasia (transitional cell hyperplasia) was noted in 6/20 high dose males and minimal urothelial hyperplasia was noted in 1/20 high dose females. Minimal urothelial hyperplasia was still noted in 1/6 high dose recovery males.

Eyes:

Lenticular alterations, consisting of small to large cataracts, minimal to slight swelling of lenticular fibers and/or occasional minimal to slight epithelial proliferation were noted in high dose animals (males: 13/20; females: 3/20). The changes were mainly bilateral. Small to large cataracts were evident in 4/6 high dose recovery males and a small cataract was noted in the right lens of 1/6 high dose recovery females.

- **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)			$AUC_{0\text{-}24hr}$ (ng•hr/ml)		
		Week 5	Week 14	Week 27	Week 5	Week 14	Week 27	Week 5	Week 14	Week 27
1	Male	7.8	9.3	1.9	0.5	0.5	1.5	19.6	31.3	17.5
	Female	3.5	10.2	6.8	0.5	0.5	0.5	18.6	49.0	23.7
5	Male	169	223	206	0.5	0.5	0.5	386	653	689
	Female	82.2	137	71.7	0.5	0.5	0.5	198	214	225
25	Male	1715	2163	1408	1.5	1.5	1.5	10923	12725	6012
	Female	2034	2423	2524	1.5	1.5	1.5	13308	14600	11368

A dose dependent increase in systemic exposure was noted in this study. No indication of drug accumulation in the blood was noted in this study. Males tended to have higher systemic exposure than females in the mid dose group. However, females tended to have higher systemic exposure than males in the high dose group.

Summary of individual study findings:

Potential target organs of toxicity identified in this study included thymus, kidneys, lungs, pancreas, sternum, spleen, mesenteric and mandibular lymph nodes, prostate, salivary glands, urinary bladder and eyes. It is interesting to note that histopathological changes in the prostate were noted after 26 weeks of treatment in the rat. Only a decrease in prostate weight was noted in previous toxicity studies in rats up to 13 weeks duration. The effects noted in the thymus, spleen and mesenteric and mandibular lymph nodes are probably related to the pharmacological (immunosuppressive) activity of ASM 981. The effects noted in all of the other potential target organs were probably related to overt toxicity associated with ASM 981. The NOAEL identified in this study was 1 mg/kg/day ($AUC_{0\text{-}24\ hr}$ = 17.5 and 23.7 ng·hr/ml for males and females, respectively) for rats after 26 weeks of oral administration of ASM 981 (solid dispersion).

Dermal Rat FMF:

Repeat Dose Toxicology Study #14:

A 13-week dermal toxicity study in rats

Study Title: A 13-week dermal toxicity study in rats
Study No: T-30/203-205

Contract Study No:	972021
Volume #, and page #:	30, 5-174
Conducting laboratory:	[redacted]
Date of study initiation:	10/15/97
GLP compliance:	Yes
QA- Report:	Yes (X) No ()
Drug, and lot#:	0.2% ASM 981 cream – batch# Z008 0297 0.6% ASM 981 cream – batch# Z010 0297 1.0% ASM 981 cream – batch# Z004 0197
Formulation/vehicle:	Vehicle cream – batch# Z002 0197

Methods:

Prior to treatment, the hair was clipped from an area on the back and flank corresponding to ~10% of the total body surface. Clipping was repeated on an as needed basis. Animals were treated daily with 1 gm/kg of vehicle or test article formulations distributed evenly over the clipped area. The treatment site was covered with a 4 layer gauze pack. The gauze pack was fixed with Micropore Tape wound round the trunk. The treated area was cleaned with soap and water and dried at the end of the 20 hour treatment period. All animals were dosed once daily, 7 days/week, for a duration of 13 weeks.

Dosing:

- *species/strain:* Wistar rats
- *#/sex/group or time point:* 10/sex/dose
- *satellite groups used for toxicokinetics or recovery:* NA
- *age:* 6 – 9 weeks
- *weight:* 118 - 167 g
- *doses in administered units:* 0 (Untreated control), 0 (Vehicle control; 0%), 2 (0.2%), 6 (0.6%) and 10 (1.0%) mg/kg/day ASM 981
- *route, form, volume, and infusion rate:* topical; cream; 1 gm/kg

Observations and times:

- *Clinical signs:*	daily
- *Dermal reactions:*	daily (after removal of test article)
- *Body weights:*	weekly
- *Food consumption:*	weekly
- *Ophthalmology:*	pretest and week 13
- *Hematology:*	week 13
- *Clinical chemistry:*	week 13
- *Urinalysis:*	week 13
- *Gross pathology:*	at sacrifice
- *Organ weights:*	adrenals, brain, heart, kidneys, liver, ovaries, pituitary, prostrate, spleen, testes, thymus, thyroid with parathyroids and uterus
- *Histopathology:*	The following organs were preserved in 10% buffered formalin from all animals: adrenals, aorta, bone (femur/tibia), brain,

cecum, colon, duodenum, epididymides, esophagus, eyes, harderian glands, heart, ileum, jejunum, kidneys, lacrimal glands, liver, lungs, lymph nodes (bronchial, mandibular, mesenteric), mammary gland, ovaries, pancreas, pituitary, prostate, rectum, salivary glands, sciatic nerve, seminal vesicles, skeletal muscle, 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 listed tissues from all animals were examined microscopically for control and high dose animals.

- *Toxicokinetics:* Blood samples were obtained from animals (2/sex/dose) at 0.5, 1, 3, 7 and 24 hours after dosing on day 85. ASM 981 levels were determined in the blood samples by [redacted] The limit of quantification was [redacted] ng/ml.

Results:

- **Clinical signs** No treatment related effects on mortality or clinical signs were noted in this study.
- **Dermal reactions** No treatment related effects on dermal reactions 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.
- **Ophthalmology** No treatment related effects on ophthalmologic parameters were noted in this study.
- **Hematology** No treatment related effects on hematological parameters were noted in this study.
- **Clinical Chemistry** No treatment related effects on clinical chemistry parameters were noted in this study.
- **Urinalysis** No treatment related effects on urinalysis parameters were noted in this study.
- **Gross pathology** No treatment related effects on macroscopic findings were noted in this study.

- **Organ weights** No treatment related effects on organ weights were noted in this study.

- **Histopathology** The skin at the application site was thickened in vehicle control and high dose animals compared to untreated control animals. The epidermal thickening consisted of minimal acanthosis in males and minimal acanthosis with hypergranularity and hyperkeratosis in females. No other treatment related effects on microscopic findings were noted in this study

- **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_{0-24hr} (ng•hr/ml)
2 (0.2%)	Male	--	--	--
	Female	--	--	--
6 (0.6%)	Male	0.9	7	11.8
	Female	0.5	7	8.0
10 (1%)	Male	0.7	24	10.4
	Female	0.8	7	15.8

ASM 981 blood concentrations were below the limit of quantitation in low dose animals. Animals in the mid and high dose group did have systemic exposure in this study, but no apparent dose proportional increase was noted in this study. No apparent difference based on sex was noted in this study.

Summary of individual study findings:

No systemic toxicity was noted in this study. The only treatment related effect noted in this study was a slight thickening of the epithelium noted in vehicle control and high dose animals. No difference in the extent of the epidermal thickening was noted between the two groups. This may have been due to the cream formulation and not attributed to ASM 981. Therefore, the NOAEL identified in this study was 10 mg/kg/day (1% ASM 981 cream; $AUC_{0-24\ hr}$ = 10.4 and 15.8 ng·hr/ml for males and females, respectively) for rats after 13 weeks of topical administration of ASM 981 cream.

It is not surprising that no systemic toxicity was noted in this study. The NOAEL identified in a 4 week oral toxicity study in rats was 2 mg/kg/day. The $AUC_{0-24\ hr}$ values for this dose were 68.5 and 36.2 ng·hr/ml for males and females, respectively. The high dose group in this 13 week rat dermal toxicity study did not yield $AUC_{0-24\ hr}$ values that were greater than the 4 week oral rat NOAEL. Therefore, no systemic toxicity would be anticipated in this study. However, the design of the study is adequate because the 1% ASM 981 cream is the maximum feasible concentration in the to be marketed cream formulation and an adequate amount of the cream was applied daily for 20 hours/day.

Repeat Dose Toxicology Study #15:

A 26-week dermal toxicity study in rats

Study Title:	A 26-week dermal toxicity study in rats
Study No:	T-31/203-216
Contract Study No:	972022
Volume #, and page #:	31, 5-1
Conducting laboratory:	[redacted]
Date of study initiation:	10/21/97
GLP compliance:	Yes
QA- Report:	Yes (X) No ()
Drug, and lot#:	0.2% ASM 981 cream – batch# Z008 0297
	0.6% ASM 981 cream – batch# Z010 0297
	1.0% ASM 981 cream – batch# Z004 0197
Formulation/vehicle:	Vehicle cream – batch# Z002 0197

Methods:

Prior to treatment, the hair was clipped from an area on the back and flank corresponding to ~10% of the total body surface. Clipping was repeated on an as needed basis. Animals were treated daily with 1 gm/kg of vehicle or test article formulations distributed evenly over the clipped area. The treatment site was covered with a 4 layer gauze pack. The gauze pack was fixed with Micropore Tape wound round the trunk. The treated area was cleaned with soap and water and dried at the end of the 20 hour treatment period. All animals were dosed once daily, 7 days/week, for a duration of 26 weeks.

Dosing:

- *species/strain:* Wistar rats
- *#/sex/group or time point:* 20/sex/dose
- *satellite groups used for toxicokinetics or recovery:* NA
- *age:* 6 – 9 weeks
- *weight:* 129 - 178 g
- *doses in administered units:* 0 (Untreated control), 0 (Vehicle control; 0%), 2 (0.2%), 6 (0.6%) and 10 (1.0%) mg/kg/day ASM 981
- *route, form, volume, and infusion rate:* topical; cream; 1 gm/kg

Observations and times:

- *Clinical signs:* daily
- *Dermal reactions:* daily (after removal of test article)
- *Body weights:* weekly
- *Food consumption:* weekly
- *Ophthalmology:* pretest, week 13 and week 26
- *Hematology:* week 13 and week 26

- *Clinical chemistry:* week 13 and week 26
- *Urinalysis:* week 13 and week 26
- *Gross pathology:* at sacrifice
- *Organ weights:* adrenals, brain, heart, kidneys, liver, ovaries, pituitary, prostrate, spleen, testes, thymus, thyroid with parathyroids and uterus
- *Histopathology:* The following organs were preserved in 10% buffered formalin from all animals: adrenals, aorta, bone (femur/tibia), brain, cecum, colon, duodenum, epididymides, esophagus, eyes, harderian glands, heart, ileum, jejunum, kidneys, lacrimal glands, liver, lungs, lymph nodes (bronchial, mandibular, mesenteric), mammary gland, ovaries, pancreas, pituitary, prostate, rectum, salivary glands, sciatic nerve, seminal vesicles, skeletal muscle, 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 listed tissues from all animals were examined microscopically for control and high dose animals.

- *Toxicokinetics:* Blood samples were obtained from animals (4/sex/dose) at 0.5, 1, 3, 7 and 24 hours after dosing in week 14 and week 26. ASM 981 levels were determined in the blood samples by —— The limit of quantification was — ng/ml.

Results:

- **Clinical signs** No treatment related effects on mortality or clinical signs were noted in this study.
- **Dermal reactions** No treatment related effects on dermal reactions 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.
- **Ophthalmology** No treatment related effects on ophthalmologic parameters were noted in this study.
- **Hematology** No treatment related effects on hematological parameters were noted in this study.

- **Clinical Chemistry** No treatment related effects on clinical chemistry parameters were noted in this study.

- **Urinalysis** No treatment related effects on urinalysis parameters were noted in this study.

- **Gross pathology** No treatment related effects on macroscopic findings were noted in this study.

- **Organ weights** No treatment related effects on organ weights were noted in this study.

- **Histopathology** Epidermal thickening, consisting of diffuse minimal/slight acanthosis, diffuse minimal hypergranularity and diffuse minimal hyperkeratosis was noted in all animals in the vehicle control and high dose animals. No difference was noted between the treatment groups. No other treatment related effects on microscopic findings were noted in this study

- **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)		$AUC_{0\text{-}24hr}$ (ng•hr/ml)	
		Week 14	Week 26	Week 14	Week 26	Week 14	Week 26
2 (0.2%)	Male	--[a]	0.33	--[a]	24	--[a]	--[b]
	Female	--[a]	0.07	--[a]	3	--[a]	--[b]
6 (0.6%)	Male	0.67	0.57	1	3	7.97	11.0
	Female	0.44	0.47	7	7	6.02	6.31
10 (1%)	Male	0.35	0.34	3	7	5.62	4.93
	Female	0.50	0.36	3	3	6.62	4.89

a – ASM 981 blood concentrations were below the limit of quantitation in low dose animals for the week 14 timepoint.
b – $AUC_{0\text{-}24\ hr}$ could not be calculated for the low dose week 26 timepoint due to a number of the samples were below the limit of quantitation.

ASM 981 blood concentrations were below the limit of quantitation in low dose animals. Animals in the mid and high dose group did have systemic exposure in this study, but no apparent dose proportional increase was noted in this study. It appeared as if the mid dose group may have had slightly higher systemic exposure than the high dose group in this study. No apparent difference based on sex was noted in this study.

Summary of individual study findings:

No systemic toxicity was noted in this study. The only treatment related effect noted in this study was a slight thickening of the epithelium noted in vehicle control and high dose

animals. No difference in the extent of the epidermal thickening was noted between the two groups. This may have been due to the cream formulation and not attributed to ASM 981. Therefore, the NOAEL identified in this study was 10 mg/kg/day (1% ASM 981 cream; $AUC_{0\text{-}24\ hr}$ = 4.9 ng·hr/ml for males and females) for rats after 26 weeks of topical administration of ASM 981 cream.

It is not surprising that no systemic toxicity was noted in this study. The NOAEL identified in a 26 week oral toxicity study in rats was 1 mg/kg/day. The $AUC_{0\text{-}24\ hr}$ values for this dose were 17.5 and 23.7 ng·hr/ml for males and females, respectively. The high dose group in this 26 week rat dermal toxicity study did not yield $AUC_{0\text{-}24\ hr}$ values that were greater than the 4 week oral rat NOAEL. Therefore, no systemic toxicity would be anticipated in this study. However, the design of the study is adequate because the 1% ASM 981 cream is the maximum feasible concentration in the to be marketed cream formulation and an adequate amount of the cream was applied daily for 20 hours/day.

Oral Minipig:

Repeat Dose Toxicology Study #16:

Two-week oral (gavage) toxicity study in the minipig (Drug form: lyophilisate suspension)

Study Title:	Two-week oral (gavage) toxicity study in the minipig (Drug form: lyophilisate suspension)
Study No:	T-40/203-042
Contract Study No:	14969
Volume #, and page #:	36, 5-1
Conducting laboratory:	[redacted]
Date of study initiation:	8/4/94
GLP compliance:	Yes
QA- Report:	Yes (X) No ()
Drug, and lot#:	ASM 981 – batch# Y194 0794
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 2 weeks.

Dosing:

- *species/strain:* Gottingen SPF minipigs
- *#/sex/group or time point:* 2/sex/dose
- *satellite groups used for toxicokinetics or recovery:* N/A
- *age:* 9 - 12 weeks
- *weight:* 5.0 – 6.9 kg

- *doses in administered units:* 0, 5, 15 and 45 mg/kg/day ASM 981
- *route, form, volume, and infusion rate:* oral; liquid suspension; 4.5 ml/kg for control and high dose groups, 0.5 ml/kg for low dose group and 1.5 ml/kg for mid dose group

Observations and times:

- *Clinical signs:*	daily
- *Body weights:*	days 1, 7 and 14
- *Food Consumption:*	daily
- *Ophthalmology:*	pretest and day 13
- *Electrocardiography:*	pretest and day 13
- *Hematology:*	pretest and day 12
- *Clinical chemistry:*	pretest and day 12
- *Urinalysis:*	pretest and day 12
- *Gross pathology:*	at sacrifice
- *Organ weights:*	Adrenals, brain, heart, kidneys, liver, pituitary gland, prostrate/uterus, spleen, testes/ovaries, thymus and thyroid glands
- *Histopathology:*	The following organs were preserved in 10% buffered formalin from all animals: adrenals, aorta, bones (femur/tibia, right), brain, esophagus, eyes, gall bladder, heart, intestine-small (duodenum, jejunum, ileum), intestine-large (cecum, colon, rectum), kidneys, liver, lungs, lymph nodes (mandibular and mesenteric), mammary glands, muscle (quadriceps femoris, right), 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 listed organs were examined microscopically from all treated animals.
- *Toxicokinetics:*	Blood samples were obtained from animals (2/sex/dose) at 0.5, 1, 2, 4, 7 and 24 hours after the last dose. ASM 981 levels were determined in the blood samples by [redacted] [redacted] The limit of quantification was — ng/ml.

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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 weights were noted in this study.
- **Food Consumption** No treatment related effects on food consumption were noted in this study.
- **Ophthalmology** No treatment related effects on ophthalmologic parameters were noted in this study.
- **Electrocardiography** No treatment related effects on electrocradiograph parameters were noted in this study.
- **Hematology** No treatment related effects on hematologic parameters were noted in this study.
- **Clinical Chemistry** No treatment related effects on clinical chemistry parameters were noted in this study.
- **Urinalysis** No treatment related effects on urinary parameters were noted in this study.
- **Gross pathology** No treatment related macroscopic findings were noted in this study.
- **Organ Weights** A significant decrease in thymus weight was noted in mid (males: ↓52.3%; females: ↓61.7%) and high dose animals (males: ↓54.2%; females: ↓50.3%).
- **Histopathology**

Note: In general, the microscopic findings are presented as a combination of male and female animals in this section due to low number of animals per sex per group (i.e. 2/sex/group)

Minimal thymic medullary atrophy was present in one high dose female. Minimal eosinophilic inflammatory cell infiltration in the mucosa of the stomach (3/4 animals), duodenum (1/4 animals), cecum (1/4 animals) and colon (1/4 animals) was noted in high dose animals. No similar infiltration was noted in animals from control, low or mid dose groups.

Minimal arterial fibrinoid necrosis in a few small arteries was observed in the cecum, colon, kidneys, skeletal muscle, ovaries, rectum, spleen, thyroid, urinary bladder and uterus of one high dose animal. Minimal arterial fibrinoid necrosis was also found in one small artery in both kidneys from another high dose animal.

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

Dose (mg/kg/day)	Sex	T_{max} (hr)	C_{max} (ng/ml)	$AUC_{0-24\ hr}$ (ng·hg/ml)
5	Male	7 ± 0	22 ± 15	320 ± 191
	Female	7 ± 0	43 ± 17	610 ± 127
15	Male	1.3 ± 1.1	145 ± 110	1482 ± 761
	Female	2.5 ± 2.1	103 ± 19	1442 ± 761
45	Male	4.5 ± 3.5	208 ± 162	2600 ± 908
	Female	1.5 ± 0.7	362 ± 216	3485 ± 1791

A dose dependent increase in ASM 981 systemic exposure was noted in this study. No apparent difference based on sex was noted in this study.

Summary of individual study findings:

Potential target organs of toxicity identified in this study included the thymus, small arteries and the mucosa of the gastrointestinal tract in minipigs. The effects noted in the thymus are probably related to the pharmacological (immunosuppressive) activity of ASM 981. Similar findings of decreased weight of the thymus and minimal thymic medullary atrophy have been noted in rats. The effects noted in the small arteries and mucosa of the gatrointestinal tract were probably related to overt toxicity associated with ASM 981. The NOAEL identified in this study was 5 mg/kg/day ($AUC_{0-24\ hr}$ = 320 and 610 ng·hr/ml for males and females, respectively) for minipigs after 2 weeks of oral administration of ASM 981 (lyophilisate suspension).

Repeat Dose Toxicology Study #17:

A 2-week oral (gavage) toxicity study in minipigs (Drug form: solid dispersion)

Study Title:	A 2-week oral (gavage) toxicity study in minipigs (Drug form: solid dispersion)
Study No:	T-43/203-126
Contract Study No:	15757
Volume #, and page #:	36, 5-132
Conducting laboratory:	[redacted]
Date of study initiation:	4/26/95
GLP compliance:	Yes
QA- Report:	Yes (X) No ()
Drug, and lot#:	ASM 981 – batch# X054 0495
Formulation/vehicle:	Aqueous solution containing 0.5% Poloxamer 188 and 3.5% hydroxypropylmethyl cellulose (Note: The stock oral ASM 981 solution was referred to as the solid dispersion and consisted of 20% ASM 981 (w/w) solid dispersion in 0.5% Poloxamer 188 and 3.5% hydoxypropylmethyl cellulose as a carrier in water.)

Methods:

Test article or vehicle (aqueous solution containing 0.5% Poloxamer 188 and 3.5% hydroxypropylmethyl cellulose) was administered orally (via gavage) on a daily basis, 7 days/week, for 2 weeks.

Dosing:

- *species/strain:* Gottingen SPF minipigs
- *#/sex/group or time point:* 2/sex/dose
- *satellite groups used for toxicokinetics or recovery:* N/A
- *age:* 10 – 13 weeks
- *weight:* 6.1 – 7.3 kg
- *doses in administered units:* 0, 5, 15 and 45 mg/kg/day ASM 981
- *route, form, volume, and infusion rate:* oral; liquid dispersion; 2.25 ml/kg for control and high dose groups, 0.25 ml/kg for low dose group and 0.75 ml/kg for mid dose group

Observations and times:

- *Clinical signs:* daily
- *Body weights:* weekly
- *Food Consumption:* daily
- *Ophthalmology:* pretest and day 13
- *Electrocardiography:* pretest and day 13
- *Hematology:* pretest and day 12
- *Clinical chemistry:* pretest and day 12
- *Urinalysis:* pretest and day 12
- *Gross pathology:* at sacrifice
- *Organ weights:* Adrenals, brain, heart, kidneys, liver, pituitary gland, prostrate/uterus, spleen, testes/ovaries, thymus and thyroid glands
- *Histopathology:* The following organs were preserved in 10% buffered formalin from all animals: adrenals, aorta, bones (femur/tibia, right), brain, esophagus, eyes, gall bladder, heart, intestine-small (duodenum, jejunum, ileum), intestine-large (cecum, colon, rectum), kidneys, liver, lungs, lymph nodes (mandibular and mesenteric), mammary glands, muscle (quadriceps femoris, right), 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 listed organs were examined microscopically from all treated animals.

- *Toxicokinetics:* Blood samples were obtained from animals (2/sex/dose) at 0.5, 1, 2, 4, 7 and 24 hours after the last dose. ASM 981 levels were

determined in the blood samples by ———————— The limit of quantification was — 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 weights were noted in this study.
- **Food Consumption** No treatment related effects on food consumption were noted in this study.
- **Ophthalmology** No treatment related effects on ophthalmologic parameters were noted in this study.
- **Electrocardiography** No treatment related effects on electrocradiograph parameters were noted in this study.
- **Hematology** No treatment related effects on hematologic parameters were noted in this study.
- **Clinical Chemistry** No treatment related effects on clinical chemistry parameters were noted in this study.
- **Urinalysis** No treatment related effects on urinary parameters were noted in this study.
- **Gross pathology** No treatment related macroscopic findings were noted in this study.
- **Organ Weights** A significant decrease in thymus weight was noted in mid dose females (↓43.4%) and high dose animals (males: ↓32.1%; females: ↓46.0%).
- **Histopathology**

Note: In general, the microscopic findings are presented as a combination of male and female animals in this section due to low number of animals per sex per group (i.e. 2/sex/group)

Minimal thymic medullary atrophy was present in one mid and one high dose animal.

Minimal arterial fibrinoid necrosis was noted in one high dose animal in a few small arteries in the colon, heart, ileum, jejunum, liver, mesenteric lymph node, ovary and urinary bladder. Minimal arterial fibrinoid necrosis was noted in another high dose animals in a few small arteries in the urinary bladder. Minimal arteritis/periarteritis was present in a few small arteries in one kidney in a control animal.

The study report states that arterial lesions, such as fibroid necrosis with subsequent arteritis/periarteritis are considered to be spontaneous in nature and unrelated to treatment. However, I would counter that perhaps ASM 981 is causing an increase in the prevalence and/or severity of this spontaneous lesion. It is difficult to interpret this possibility under the conditions of this study since only 2 animals/sex/dose were treated for only 2 weeks. Studies with longer a duration of treatment and larger numbers of animals per dose group may provide additional information to form a final conclusion. In general, if a toxic effect is noted in the high dose group only, then it is considered drug related until other information is received to dispute this belief.

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

Dose (mg/kg/day)	Sex	T_{max} (hr)	C_{max} (ng/ml)	$AUC_{0\text{-}24\ hr}$ (ng·hg/ml)
5	Male	14	63.0	819
	Female	4	55.5	677
15	Male	2.5	194	1903
	Female	1.5	247	2518
45	Male	1	182	1592
	Female	1	180	1292

An approximate dose dependent increase in ASM 981 systemic exposure was noted between low and mid dose groups in this study. An apparent plateau of systemic exposure was noted between the mid and high dose groups in this study. No apparent difference based on sex was noted in this study.

Summary of individual study findings:

Potential target organs of toxicity identified in this study included the thymus and small arteries in minipigs. The effects noted in the thymus are probably related to the pharmacological (immunosuppressive) activity of ASM 981. Similar findings of decreased weight of the thymus and minimal thymic medullary atrophy have been noted in rats. The effects noted in the small arteries may have been related to overt toxicity associated with ASM 981. Apparently these effects in small arteries is a spontaneous lesion in this species of minipigs. ASM 981 may have caused an increase in the incidence and/or severity of this lesion. Additional studies with a longer duration and increased number of animals per dose group may provide enough data to draw a final conclusion. The NOAEL identified in this study was 5 mg/kg/day ($AUC_{0\text{-}24\ hr}$ = 819 and 677 ng·hr/ml for males and females, respectively) for minipigs after 2 weeks of oral administration of ASM 981 (solid dispersion).