

Repeat Dose Toxicology Study #18:

A 2-week oral dose range finding study in juvenile minipigs (Drug form: solid dispersion)

Study Title: A 2-week oral dose range finding study in juvenile minipigs (Drug form: solid dispersion)
Study No: T-44/203-154
Contract Study No: 16837
Volume #, and page #: 37, 5-1
Conducting laboratory: _____
Date of study initiation: 2/15/96
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% hydroxypropylmethyl 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:* 1/sex/dose
- *satellite groups used for toxicokinetics or recovery:* N/A
- *age:* 6 weeks
- *weight:* 3.8 – 4.8 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:* day 0, 3, 7 and 10
- *Food Consumption:* daily
- *Hematology:* day 13
- *Clinical chemistry:* day 13
- *Gross pathology:* at sacrifice
- *Organ weights:* Adrenals, brain, heart, kidneys, liver, pituitary gland, prostate/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 (1/sex/dose) at 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.
- **Hematology** No treatment related effects on hematologic parameters were noted in this study.
- **Clinical Chemistry** Serum magnesium levels were significantly decreased in high dose animals (males: ↓28.9%; females: ↓41.1%). It is interesting to note that the study report states that hypomagnesaemia may be caused by drugs with an immunosuppressive action.
- **Gross pathology** No treatment related macroscopic findings were noted in this study.
- **Organ Weights** No treatment related effects on organ weights were noted in this study.
- **Histopathology** No treatment related microscopic findings were noted in this study.

- **Toxicokinetics** A summary of the toxicokinetic parameters from 1 animal/sex/dose is provided in the following table.

Dose (mg/kg/day)	Sex	T _{max} (hr)	C _{max} (ng/ml)	AUC _{0-24 hr} (ng·hr/ml)
5	Male	24	21.2	450
	Female	1	55.3	827
15	Male	2	87.5	866
	Female	1	56.0	694
45	Male	1	88.5	1164
	Female	2	485.3	4229

Since only one animal/sex/dose was treated in this study, it is difficult to draw any definitive conclusions concerning the toxicokinetic data obtained from these animals. The main thing to be stated is that systemic exposure did occur in this study.

Summary of individual study findings:

Since only one animal/sex/dose was treated in this study, it is difficult to draw any definitive conclusions from the data obtained in this study. The only treatment related effect noted in this study was a significant decrease in magnesium in high dose animals. This effect was also noted in rats. It is interesting to note that the study report states that hypomagnesemia may be caused by drugs with an immunosuppressive action. This effect was not noted in the two previous studies conducted in older minipigs. This may indicate that younger minipigs may be more sensitive to this effect. The NOAEL identified in this study was 15 mg/kg/day (AUC_{0-24 hr} = 866 and 694 ng·hr/ml for males and females, respectively) for juvenile minipigs after 2 weeks of oral administration of ASM 981 (solid dispersion).

Repeat Dose Toxicology Study #19:

4-week oral (gavage) toxicity study in juvenile minipigs (Drug form: solid dispersion)

Study Title: 4-week oral (gavage) toxicity study in juvenile minipigs (Drug form: solid dispersion)
Study No: T-46/203-137
Contract Study No: 17134
Volume #, and page #: 37, 5-132
Conducting laboratory: _____
Date of study initiation: 4/25/96
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% hydroxypropylmethyl 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. Recovery animals were maintained for a 2 week treatment free period after 4 weeks of dosing.

Dosing:

- *species/strain:* Gottingen SPF minipigs
- *#/sex/group or time point:* 3/sex/dose
- *satellite groups used for toxicokinetics or recovery:* Recovery – 2/sex/dose for control and high dose groups
- *age:* 7 - 8 weeks
- *weight:* 3.9 – 4.2 kg
- *doses in administered units:* 0, 5, 15 and 45 mg/kg/day ASM 981
- *route, form, volume, and infusion rate:* oral; solid 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:* twice weekly
- *Food Consumption:* daily
- *Ophthalmology:* pretest and week 4
- *Electrocardiography:* pretest and week 4
- *Hematology:* pretest, week 4 and week 6 (recovery animals)
- *Clinical chemistry:* pretest, week 4 and week 6 (recovery animals)
- *Urinalysis:* pretest, week 4 and week 6 (recovery animals)
- *Gross pathology:* at sacrifice
- *Organ weights:* Adrenals, brain, heart, kidneys, liver, pituitary gland, prostate/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 (3/sex/dose) at 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 were noted in this study. Clinical signs of depression and diarrhea were noted in high dose animals. The clinical signs became apparent during the last week of treatment. The clinical signs were not apparent in recovery animals.
- **Body weights** Body weight was significantly decreased in high dose animals (males: ↓22.0%; females: ↓12.1%) compared to control animals at the end of the treatment period.

Body weight returned to normal in high dose recovery animals.
- **Food Consumption** Food consumption was slightly decreased in high dose animals compared to control animals. Food consumption returned to normal in high dose recovery animals.
- **Ophthalmology** No treatment related effects on ophthalmologic parameters were noted in this study.
- **Electrocardiography** No treatment related effects on electrocardiograph parameters were noted in this study.
- **Hematology** No treatment related effects on hematologic parameters were noted in this study.
- **Clinical Chemistry** Serum calcium levels were decreased in high dose animals (males: ↓4.7%; females: ↓26.7%). Serum magnesium levels were decreased in high dose animals (males: ↓21.1%; females: ↓13.8%). Serum phosphorus levels were decreased in high dose animals (males: ↓21.0%; females: ↓11.4%). Serum albumin levels were decreased in high dose animals (males: ↓27.5%; females: ↓33.1%).

The effected clinical chemistry parameters returned to normal in high dose recovery animals.
- **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 high dose animals (males: ↓70.0%; females: ↓51.4%). A significant increase in adrenal weight was noted in high dose animals (males: ↑1.3X; females: ↑1.5X).

A significant decrease in thymus weight was still noted in high dose recovery animals (males: ↓65.5%; females: ↓40.4%). A significant increase in adrenal weight was noted in high dose recovery males only (↑1.4X).
- **Histopathology** Treatment related findings were noted in the adrenals, thymus and small arteries after 4 weeks of treatment. Microscopic findings were not noted in recovery animals.

Adrenals:

Focal or diffuse distribution of lymphoid follicles in the cortex was noted in mid dose females (1/3) and high dose animals (males: 2/3; females: 1/3). Similar lymphoid cell infiltrations were noted in the high dose recovery group (males: 1/2; females: 1/2).

Thymus:

Cortical atrophy was noted in high dose animals (males: 1/3; females: 2/3). Medullary atrophy was noted in high dose animals (males 1/3; females: 1/3). Medullary atrophy was also noted in both high dose recovery male animals and one high dose recovery female. One of the high dose recovery males also had cortical atrophy.

Small arteries:

A minimal fibrinoid necrotizing arteritis was present in 1/3 high dose males. This effect was not noted in high dose recovery animals.

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

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Dose (mg/kg/day)	Sex	T _{max} (hr)	C _{max} (ng/ml)	AUC _{0-24 hr} (ng·hr/ml)
5	Male	6 ± 2	31 ± 10	545 ± 242
	Female	1 ± 0	60 ± 27	444 ± 178
15	Male	17 ± 8	255 ± 125	2641 ± 470
	Female	2 ± 1	216 ± 53	1847 ± 248
45	Male	4 ± 2	198 ± 107	2445 ± 1087
	Female	1 ± 0	348 ± 168	2297 ± 985

A high interindividual variability in systemic exposure was noted in this study. A significant underproportionality was noted between doses and AUC levels. An apparent plateau was noted between the mid and high dose groups. No apparent difference in systemic exposure based on sex was noted in this study.

Summary of individual study findings:

Potential target organs of toxicity identified in this study included the adrenals, thymus and small arteries in minipigs. The effects noted in the adrenals and 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. This effect was noted in the 2 week repeat dose oral toxicity study in adult minipigs but not in a 4 week repeat dose oral toxicity study in adult minipigs. The NOAEL identified in this study was 5 mg/kg/day (AUC_{0-24 hr} = 545 and 444 ng·hr/ml for males and females, respectively) for juvenile minipigs after 4 weeks of oral administration of ASM 981 (solid dispersion).

Repeat Dose Toxicology Study #20:

4-week oral (gavage) toxicity study in minipigs (Drug form: lyophilisate suspension)

Study Title: 4-week oral (gavage) toxicity study in minipigs (Drug form: lyophilisate suspension)
Study No: T-47/203-047
Contract Study No: 15083
Volume #, and page #: 38, 5-1
Conducting laboratory: _____
Date of study initiation: 10/21/94
GLP compliance: Yes
QA- Report: Yes (X) No ()
Drug, and lot#: ASM 981 – batch# Y272 0994, Y288 1094, Y289 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 4 weeks. Recovery animals were maintained for a 4 week treatment free period after 4 weeks of dosing.

Dosing:

- *species/strain:* Gottingen SPF minipigs
- *#/sex/group or time point:* 3/sex/dose
- *satellite groups used for toxicokinetics or recovery:* Recovery – 2/sex/dose for control and high dose groups
- *age:* 3 – 4 months
- *weight:* 5.4 – 7.4 kg
- *doses in administered units:* 0, 10, 30 and 60 mg/kg/kg ASM 981
- *route, form, volume, and infusion rate:* oral; liquid suspension; 6.0 ml/kg for control and high dose groups, 1.0 ml/kg for low dose group and 03.0 ml/kg for mid dose group

Observations and times:

- *Clinical signs:* daily
- *Body weights:* weekly
- *Food Consumption:* daily
- *Ophthalmology:* pretest and week 4
- *Electrocardiography:* pretest and week 4
- *Hematology:* pretest, week 4 and week 6 (recovery animals)
- *Clinical chemistry:* pretest, week 4 and week 6 (recovery animals)
- *Urinalysis:* pretest, week 4 and week 6 (recovery animals)
- *Gross pathology:* at sacrifice
- *Organ weights:* Adrenals, brain, heart, kidneys, liver, pituitary gland, prostate/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 (3/sex/dose) at 1, 2, 4, 7 and 24 hours after the last dose. A skin sample of ~4 x 4 cm was taken for analysis from the dorsal back area of each ASM 981 treated animal 24 hours after the last dose. ASM 981 levels were determined in the blood samples by _____ The limit of quantification was _____ ng/ml. ASM 981 levels were determined in the skin samples by _____
_____ The limit of quantification was _____ ng/g.

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 electrocardiograph parameters were noted in this study.
- **Hematology** No treatment related effects on hematologic parameters were noted in this study.
- **Clinical Chemistry** Serum magnesium levels were slightly decreased in mid dose animals (males: ↓16.7%; females: ↓21.1%) and high dose males (↓21.0%).
The effected clinical chemistry parameter returned to normal in high dose recovery animals.
- **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 decrease in thymus weight was noted in mid dose animals (males: ↓45.8%; females: ↓20.0%) and high dose animals (males: ↓11.6%; females: ↓22.2%). No decrease in thymus weights was noted in recovery animals.

- **Histopathology** Low grade erosion/ulceration of the nonglandular stomach was noted in high dose animals (males: 2/3; females; 1/3). These lesions were not present in recovery animals. No other treatment related microscopic findings were noted in this study.
- **Toxicokinetics** A summary of the toxicokinetic (mean) parameters is provided in the following table. The study report provided the toxicokinetic parameters for male and female animals combined in this study.

Dose (mg/kg/day)	T _{max} (hr)	C _{max} (ng/ml)	AUC _{0-24 hr} (ng·hr/ml)
10	4	83	1129
30	3	112	1692
60	3	195	2446

A dose dependent increase in ASM 981 systemic exposure was noted in this study.

The average skin concentrations (mean ± SD) 24 hours after the last dose are provided in the following table.

Dose (mg/kg/day)	Male Conc. (ng/g)	Female Conc. (ng/g)	M + F Conc. (ng/g)
10	177 ± 80	155 ± 60	166 ± 64
30	369 ± 278	525 ± 32	447 ± 196
60	656 ± 241	728 ± 152	692 ± 184

The skin concentrations of ASM 981 increased in a dose proportional manner. No apparent sex difference was observed in this study. A comparison of the skin concentration to the C_{max} concentration obtained at each dose level demonstrates a skin/blood ratio that ranged between 2.5 – 4.4. This result indicates that ASM 981 concentration in the skin was higher than the maximal concentration in the blood.

Summary of individual study findings:

The only potential target organ of toxicity identified in this study histopathologically was the glandular stomach in minipigs. Thymus weights were reduced in this study but there was no corresponding histopathological correlate. The results of this study are in contrast to previous studies, which showed histopathological effects in the adrenals and thymus after oral ASM 981 treatment. In addition, no effects of the small arteries were noted in this study. It is unclear why the results of this study are so different than previously conducted studies in minipigs. The NOAEL identified in this study was 5 mg/kg/day (AUC_{0-24 hr} = 1129 ng·hr/ml) for minipigs after 4 weeks of oral administration of ASM 981 (liquid suspension). It is interesting to note that toxicokinetic results from this study demonstrated that ASM 981 partitions to the skin in relatively high concentrations after oral administration (2.5 – 4.4 fold higher levels than C_{max} levels in the blood).

Repeat Dose Toxicology Study #21:

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

Study Title: A 26-week oral (gavage) toxicity study in minipigs (Drug form: solid dispersion)
Study No: T-50/203-135
Contract Study No: 16226
Volume #, and page #: 39, 5-1
Conducting laboratory: _____
Date of study initiation: 3/25/96
GLP compliance: Yes
QA- Report: Yes (X) No ()
Drug, and lot#: ASM 981 – batch# X105 0695 and X147 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% hydroxypropylmethyl 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. Recovery animals were maintained for a 4 week treatment free period after 26 weeks of dosing.

Dosing:

- *species/strain:* Gottingen SPF minipigs
- *#/sex/group or time point:* 5/sex/dose
- *satellite groups used for toxicokinetics or recovery:* Recovery – 2/sex/dose for control and high dose groups
- *age:* 12 – 18 weeks
- *weight:* 6.2 – 8.1 kg
- *doses in administered units:* 0, 2, 8 and 30 mg/kg/day ASM 981
- *route, form, volume, and infusion rate:* oral; solid dispersion; 1.50 ml/kg for control and high dose groups, 0.10 ml/kg for low dose group and 0.40 ml/kg for mid dose group

Observations and times:

- *Clinical signs:* daily
- *Body weights:* weekly
- *Food Consumption:* weekly
- *Ophthalmology:* pretest, week 13 and week 26
- *Electrocardiography:* pretest, week 6, week 13 and week 26
- *Hematology:* pretest, week 6, week 13, week 26 and week 30 (recovery animals)

- *Clinical chemistry:* pretest, week 6, week 13, week 26 and week 30 (recovery animals)
- *Urinalysis:* pretest, week 6, week 13, week 26 and week 30 (recovery animals)
- *Gross pathology:* at sacrifice
- *Organ weights:* Adrenals, brain, heart, kidneys, liver, pituitary gland, prostate/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 (5/sex/dose) at 0, 1, 2, 4, 7 and 24 hours after the first and last dose. ASM 981 levels were determined in the blood samples by
The limit of quantification was — ng/ml.

Results:

- **Clinical signs** Six high dose animals (3 males and 3 females) died or were scarified before the end of the 26 week treatment period. Loose stools or diarrhea was noted sporadically in two control animals, three low dose animals, two mid dose animals and four high dose animals.
- **Body weights** Body weight gain was reduced in high dose animals after 13 weeks (males: ↓40%; females: ↓29%) and 26 weeks of treatment (males: ↓40%; females: ↓11%) compared to control animals. Body weight gain was not affected in low and mid dose animals. Body weight gain returned to normal in high dose recovery animals.
- **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 electrocardiograph parameters were noted in this study.
- **Hematology** No treatment related effects on hematologic parameters were noted in this study.
- **Clinical Chemistry** The following effects on clinical chemistry parameters were noted after 26 weeks of treatment. It is important to note that similar effects were noted after 6 and 13 weeks of treatment. Serum magnesium levels were decreased in mid (males: ↓24.4%; females: ↓15.5%) and high dose animals (males: ↓39.2%; females: ↓24.7%). Serum phosphorus levels were decreased in mid dose animals (males: ↓13.9%; females: ↓8.9%) and high dose males (↓22.7%).

The effected clinical chemistry parameters returned to normal in high dose recovery animals.
- **Urinalysis** No treatment related effects on urinary parameters were noted in this study.
- **Gross pathology** Enlarged adrenals were noted in two high dose males and one high dose female.
- **Organ Weights** An increase in adrenal weight was noted in high dose animals (males: ↑1.3X; females: ↑1.2X) compared to control animals. A significant decrease in thymus weight was noted in mid dose females (↓31.4%) and high dose animals (males: ↓25.8%; females: ↓37.4%) compared to control animals.

No increase in adrenal weight was noted in high dose recovery animals. A significant decrease in thymus weight was still noted in high dose recovery animals (males: ↓50.3%; females: ↓35.0%) compared to control animals.
- **Histopathology** Treatment related findings were noted in the arteries (arteritis/fibrinoid necrosis), adrenals (lymphoid follicles in zona glomerulosa/medulla) and lung (increased bronchi-associated-lymphoid-tissue).

Arteries:

The study report presents the findings of arteritis in combined main study and recovery animals due to the high mortality noted in the high dose group. Minimal to marked fibroid necrotizing arteritis was present in high dose animals (males: 4/7; females: 3/7). Except for one animal, this change was confined to the animals, which died or were sacrificed moribund. The

location of the arteritis was in the adrenals of all animals and in various other organs in some of these animals. The study report states that the cause of death was considered to be the arteritis.

Adrenals:

Focal distribution of lymphocytes in the zona glomerulosa was noted in mid dose animals (males: 3/5; females: 4/5). Focal/diffuse distribution of lymphocytes in the zona glomerulosa/medulla was noted in high dose animals (males: 5/5; females: 5/5). Focal distribution of lymphocytes in the zona glomerulosa was still present in the high dose recovery animals.

The moderate to marked fibrinoid necrotizing arteritis in the adrenals noted in high dose animals was accompanied by slight to marked focal cortical necrosis (males: 3/5; females: 4/5) and in some cases with severe mineralization.

Lungs:

A minimal to slight increase in bronchi-associated-lymphoid tissue was noted in mid (males: 2/5; females: 2/5) and high dose animals (males: 4/5; females: 3/5). A minimal increase in bronchi-associated-lymphoid tissue was still present in high dose recovery animals.

- **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 _{0-24 hr} (ng·hr/ml)	
		Day 1	Day 180	Day 1	Day 180	Day 1	Day 180
2	Male	7	4	16	30	152	316
	Female	3	3	13	28	147	305
8	Male	3	4	100	116	956	1552
	Female	4	4	102	98	920	1133
30	Male	3	2	454	183	4983	2455
	Female	3	10	402	145	3878	2641

An approximate dose dependent increase in ASM 981 systemic exposure was noted in this study. A possible slight potential for drug accumulation over the six month treatment period was noted in low (~1.2X) and mid dose groups (males: 1.6X; females: 1.2X) but was not apparent in the high dose group. 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 arteries, adrenals and lungs in minipigs. Arteries were considered the major target organ for toxicity in minipigs. Damage to the arteries in the adrenals was noted in all high dose animals and in various other organs in some of the high dose animals. Effects in adrenals and lungs were noted in mid and

high dose animals. The effects noted in the adrenals and lungs were probably related to the exaggerated pharmacology of ASM 981. It is stated in the study report that the arteritis could be caused by a bacterial infection spread in the vascular system as a sequela due to immunosuppression. This is an interesting hypothesis. However, until additional data is made available to support this theory, the noted arteritis will be considered a drug related effect. It would appear that arteritis became more prominent in minipigs after treatment with ASM 981 for a longer duration of treatment. The NOAEL identified in this study was 2 mg/kg/day ($AUC_{0-24 \text{ hr}} = 316$ and $305 \text{ ng}\cdot\text{hr}/\text{ml}$ for males and females, respectively) for minipigs after 26 weeks of oral administration of ASM 981 (solid dispersion).

Dermal Minipig FMF:

Repeat Dose Toxicology Study #22:

A 4-week dermal toxicity study in minipigs

Study Title: A 4-week dermal toxicity study in minipigs
Study No: T-51/203-166
Contract Study No: 19630
Volume #, and page #: 40, 5-1
Conducting laboratory: _____
Date of study initiation: 10/3/96
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# Z048 0796
Formulation/vehicle: Vehicle cream – batch# Z036 0796

Methods:

Prior to treatment, the hair was clipped from a dorsal area (corresponding to ~20% of body surface) of the trunk of the minipigs. Clipping was repeated on an as needed basis. Animals were treated daily with 2 gm/kg of vehicle or test article formulations distributed evenly over the clipped area. The treatment site was held in contact with the skin with a porous gauze dressing that was retained by a netlike body stocking. 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 4 weeks. Recovery animals were maintained for a 2 week treatment free period after 4 weeks of dosing.

Dosing:

- *species/strain:* Gottingen SPF minipigs
- *#/sex/group or time point:* 3/sex/dose
- *satellite groups used for toxicokinetics or recovery:* Recovery – 2/sex/dose for control and high dose groups
- *age:* 3 – 4 months

- *weight*: 6.0 – 8.0 kg
- *doses in administered units*: 0 (Untreated control), 0 (Vehicle control; 0%), 4 (0.2%), 12 (0.6%) and 20 (1.0%) mg/kg/day ASM 981
- *route, form, volume, and infusion rate*: topical; cream; 2 gm/kg

Observations and times:

- *Clinical signs*: daily
- *Dermal reactions*: daily (after removal of test article)
- *Body weights*: weekly
- *Food consumption*: daily
- *Ophthalmology*: pretest and week 4
- *Electrocardiography*: pretest, week 4, and week 6 (recovery animals)
- *Hematology*: pretest, week 4, and week 6 (recovery animals)
- *Clinical chemistry*: pretest, week 4, and week 6 (recovery animals)
- *Urinalysis*: pretest, week 4, and week 6 (recovery animals)
- *Gross pathology*: at sacrifice
- *Organ weights*: adrenals, brain, heart, kidneys, liver, ovaries, pituitary, prostate, spleen, testes, thymus, thyroid 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, gall bladder, 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, tongue, trachea, urinary bladder, uterus with cervix, vagina and all gross lesions.

All listed tissues from all animals were examined microscopically.

- *Toxicokinetics*: Blood samples were obtained from animals (3/sex/dose) at 0, 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.
- **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.
- **Electrocardiography** No treatment related effects on electrocardiograph 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** No treatment related effects on microscopic findings were noted in this study.
- **Toxicokinetics** A summary of the toxicokinetic (mean \pm SD) parameters after 28 days is provided in the following table.

Dose (mg/kg/day)	Sex	C _{max} (ng/ml)	T _{max} (hr)	AUC _{0-24hr} (ng•hr/ml)
4 (0.2%)	Male	0.50 \pm 0.43	3.0 \pm 3.6	5.5 \pm 6.1
	Female	0.48 \pm 0.64	2.0 \pm 0.0	3.0 \pm 3.1
12 (0.6%)	Male	0.42 \pm 0.06	7.7 \pm 10.7	4.8 \pm 1.8
	Female	0.80 \pm 0.81	0.3 \pm 0.6	6.9 \pm 4.6
20 (1%)	Male	0.73 \pm 0.73	0.7 \pm 1.2	6.4 \pm 4.1
	Female	1.05 \pm 0.77	1.7 \pm 1.2	13.7 \pm 10.3

Low level systemic exposure was noted in all dose groups. 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 or local dermal toxicity was noted in this study. Therefore, the NOAEL identified in this study was 20 mg/kg/day (1% ASM 981 cream; $AUC_{0-24 \text{ hr}} = 6.4$ and 13.7 ng-hr/ml for males and females, respectively) for minipigs after 4 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 2 week oral toxicity study in minipigs was 5 mg/kg/day. The $AUC_{0-24 \text{ hr}}$ values for this dose were 819 and 677 ng-hr/ml for males and females, respectively. The high dose group in this 4 week minipig dermal toxicity study did not yield $AUC_{0-24 \text{ hr}}$ values that were greater than the 2 week oral minipig 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 #23:*A 13-week dermal toxicity study in juvenile minipigs*

Study Title: A 13-week dermal toxicity study in juvenile minipigs
Study No: T-52/203-191
Contract Study No: 22090
Volume #, and page #: 41, 5-1
Conducting laboratory: _____
Date of study initiation: 5/21/97
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 a dorsal area (corresponding to ~20% of body surface) of the trunk of the minipigs. Clipping was repeated on an as needed basis. Animals were treated daily with 2 gm/kg of vehicle or test article formulations distributed evenly over the clipped area. The treatment site was held in contact with the skin with a porous gauze dressing that was retained by a netlike body stocking attached to a neck collar. 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. Recovery animals were maintained for a 4 week treatment free period after 13 weeks of dosing.

Dosing:

- *species/strain*: Gottingen SPF minipigs
- *#/sex/group or time point*: 4/sex/dose
- *satellite groups used for toxicokinetics or recovery*: Recovery – 2/sex/dose for control and high dose groups
- *age*: 43 – 53 days
- *weight*: 2.3 – 5.7 kg
- *doses in administered units*: 0 (Untreated control), 0 (Vehicle control; 0%), 4 (0.2%), 12 (0.6%) and 20 (1.0%) mg/kg/day ASM 981
- *route, form, volume, and infusion rate*: topical; cream; 2 gm/kg

Observations and times:

- *Clinical signs*: daily
- *Dermal reactions*: daily (after removal of test article)
- *Body weights*: weekly
- *Food consumption*: daily
- *Ophthalmology*: pretest, week 6 and week 13
- *Electrocardiography*: pretest, week 6 and week 13
- *Hematology*: pretest, week 6, week 13 and week 17 (recovery animals)
- *Clinical chemistry*: pretest, week 6, week 13 and week 17 (recovery animals)
- *Urinalysis*: pretest, week 6, week 13 and week 17 (recovery animals)
- *Gross pathology*: at sacrifice
- *Organ weights*: adrenals, brain, heart, kidneys, liver, ovaries, pituitary, prostate, spleen, testes, thymus, thyroid 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, gall bladder, 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, tongue, trachea, urinary bladder, uterus with cervix, vagina and all gross lesions.

All listed tissues from all animals were examined microscopically.

- *Toxicokinetics*: Blood samples were obtained from animals (4/sex/dose) at 0, 1, 2, 4, 7 and 24 hours after the first and 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.
- **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.
- **Electrocardiography** No treatment related effects on electrocardiograph 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** No treatment related effects on microscopic findings were noted in this study.
- **Toxicokinetics** ASM 981 blood concentrations were not measurable on day 1 in the low dose group. A concentration close to the limit of quantitation was noted at one time point (7 hours) for one animal in the low dose group after 13 weeks of treatment.

ASM 981 blood concentrations were below the limit of quantitation on day 1 in the mid dose group. Three animals in the mid dose group showed a few measurable concentrations close to the limit of quantitation after 13 weeks of treatment.

Two ASM 981 blood concentrations were above the limit of quantitation in one high dose animal on day 1. Measurable concentrations of ASM 981 ranging from _____ ng/ml were obtained for a few animals in the high dose group after 13 weeks of treatment.

Pharmacokinetic parameters could not be determined for any animals in this study due to the low concentrations of ASM 981.

Summary of individual study findings:

No systemic or local dermal toxicity was noted in this study. Therefore, the NOAEL identified in this study was 20 mg/kg/day (1% ASM 981 cream) for juvenile minipigs after 13 weeks of topical administration of ASM 981 cream. It is unclear why no consistent systemic absorption was noted in juvenile minipigs after 13 weeks of repeat dose administration of ASM 981 cream but was noted after 4 weeks of repeat dose topical exposure to ASM 981 cream in minipigs.

It is not surprising that no systemic toxicity was noted in this study since basically no systemic exposure or very low levels of systemic exposure was noted in this study. 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 #24:

A 26-week dermal toxicity study in minipigs

<u>Study Title:</u>	A 26-week dermal toxicity study in minipigs
<u>Study No:</u>	T-53/203-190
<u>Contract Study No:</u>	20447
<u>Volume #, and page #:</u>	42, 5-1
<u>Conducting laboratory:</u>	_____
<u>Date of study initiation:</u>	1/29/97
<u>GLP compliance:</u>	Yes
<u>QA- Report:</u>	Yes (X) No ()
<u>Drug, and lot#:</u>	0.2% ASM 981 cream – batch# Z081 1096 0.6% ASM 981 cream – batch# Z083 1096 1.0% ASM 981 cream – batch# Z084 1096
<u>Formulation/vehicle:</u>	Vehicle cream – batch# Z087 1096

Methods:

Prior to treatment, the hair was clipped from a dorsal area (corresponding to ~20% of body surface) of the trunk of the minipigs. Clipping was repeated on an as needed basis. Animals were treated daily with 2 gm/kg of vehicle or test article formulations distributed evenly

over the clipped area. The treatment site was held in contact with the skin with a porous gauze dressing that was retained by a netlike body stocking. 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. Recovery animals were maintained for a 4 week treatment free period after 26 weeks of dosing.

Dosing:

- *species/strain*: Gottingen SPF minipigs
- *#/sex/group or time point*: 4/sex/dose
- *satellite groups used for toxicokinetics or recovery*: Recovery – 2/sex/dose for control and high dose groups
- *age*: 11 – 15 weeks
- *weight*: 5.5 – 7.8 kg
- *doses in administered units*: 0 (Untreated control), 0 (Vehicle control; 0%), 4 (0.2%), 12 (0.6%) and 20 (1.0%) mg/kg/day ASM 981
- *route, form, volume, and infusion rate*: topical; cream; 2 gm/kg

Observations and times:

- *Clinical signs*: daily
- *Dermal reactions*: daily (after removal of test article)
- *Body weights*: weekly
- *Food consumption*: daily
- *Ophthalmology*: pretest, week 13 and week 26
- *Electrocardiography*: pretest, week 13 and week 26
- *Hematology*: pretest, week 6, week 13, week 26 and week 30 (recovery animals)
- *Clinical chemistry*: pretest, week 6, week 13, week 26 and week 30 (recovery animals)
- *Urinalysis*: pretest, week 6, week 13, week 26 and week 30 (recovery animals)
- *Gross pathology*: at sacrifice
- *Organ weights*: adrenals, brain, heart, kidneys, liver, ovaries, pituitary, prostate, spleen, testes, thymus, thyroid 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, gall bladder, 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, tongue, trachea, urinary bladder, uterus with cervix, vagina and all gross lesions.

All listed tissues from all animals were examined microscopically.

- *Toxicokinetics:* Blood samples were obtained from animals (4/sex/dose) at 0, 1, 2, 4, 7 and 24 hours after the first dose and during week 13 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.
- **Electrocardiography** No treatment related effects on electrocradiograph 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** No treatment related effects on microscopic findings were noted in this study.

- **Toxicokinetics**

ASM 981 blood concentrations were all below the limit of quantitation after the first dose in all dose groups.

All animals but one in the low dose group had blood concentrations below the limit of quantitation in week 13 and 26 of dosing.

Low concentrations of ASM 981 were measurable in mid dose animals during week 13 but not during week 26. $AUC_{0-24 \text{ hr}}$ levels during week 13 were 85.2 and 44.6 ng-hr/ml for males and female, respectively. $AUC_{0-24 \text{ hr}}$ levels during week 26 could not be calculated due to the inconsistent and low levels of ASM 981 measured at this time point.

Low concentrations of ASM 981 were measurable in high dose animals during week 13 and week 26. $AUC_{0-24 \text{ hr}}$ levels during week 13 were 134.4 and 4.9 ng-hr/ml for males and female, respectively. $AUC_{0-24 \text{ hr}}$ levels during week 26 were 7.2 and 2.8 ng-hr/ml for males and female, respectively.

It is unclear why lower $AUC_{0-24 \text{ hr}}$ levels were noted in mid and high dose animals in the mid and high dose group after 26 weeks of treatment compared to 13 weeks of treatment.

Summary of individual study findings:

No systemic or local dermal toxicity was noted in this study. Therefore, the NOAEL identified in this study was 20 mg/kg/day (1% ASM 981 cream; $AUC_{0-24 \text{ hr}} = 7.2$ and 2.8 ng-hr/ml for males and females, respectively) for minipigs 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 minipigs was 2 mg/kg/day. The $AUC_{0-24 \text{ hr}}$ values for this dose were 316 and 305 ng-hr/ml for males and females, respectively. The high dose group in this 26 week minipig dermal toxicity study did not yield $AUC_{0-24 \text{ hr}}$ values that were greater than the 26 week oral minipig 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.

Toxicology summary:

Oral and dermal repeat dose toxicity studies were conducted in mice (duration up to 13 weeks), rats (duration up to 26 weeks) and minipigs (duration up to 26 weeks). Results from the longest duration studies and studies designed to address a particular question will be described in this summary. For additional information, the reader is referred to the review of the individual toxicity study.

The results of the 13 week oral toxicity study conducted in mice was able to delineate potential target organs for ASM 981. Doses of 0, 10, 50, 100 and 312.5 mg/kg/day ASM 981 were administered in this study. 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 ($AUC_{0.5-4hr} = 1029$ and 2949 ng·hg/ml in males and females, respectively) for mice after 13 weeks of oral administration of ASM 981.

Two dermal toxicity studies conducted in hairless mice with the final to be marketed ASM 981 cream formulation were used to support the dose range selected for the photocarcinogenicity study. A 4 week repeat dose toxicokinetic study was able to determine that significant systemic exposure to ASM 981 was achieved after dermal administration of the 1% ASM 981 cream formulation. An 8 week repeat dose phototoxicity dose range finding study was conducted in hairless mice demonstrated that the highest concentration of the ASM 981 cream (1%) was well tolerated and could be used in the photocarcinogenicity study. The rationale for dose selection in the photocarcinogenicity study will be summarized under the carcinogenicity section of this review.

The dermal toxicity studies conducted in mice used ethanol as a vehicle. These studies were conducted prior to submission of the IND to the division. The rationale for ethanol was that the dermal carcinogenicity study that was conducted for ASM 981 was conducted with ethanol as a vehicle as well. The sponsor was informed when the IND was submitted for the ASM 981 cream that it was recommended that nonclinical dermal toxicity studies be conducted with the final to be marketed cream formulation of ASM 981. Therefore, the dermal studies conducted in rats and minipigs were conducted with the final to be marketed ASM 981 cream formulation. It is important to note that the maximum feasible concentration of ASM 981 in the cream formulation is 1% and this concentration was used as the high dose in the rat and minipig dermal toxicity studies.

Dermal administration of ASM 981 in ethanol to mice for 13 weeks established the lymphoproliferative potential of ASM 981. Pleomorphic lymphoma was noted at the 60 mg/kg/day dose level. 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.

A series of dermal toxicity studies were conducted in mice to better clarify the timing of the development of lymphoproliferative lesions after topical ASM 981 administration. The focus of one 13 week dermal toxicity study in mice was to assess the dose response relationship of immunosuppression and lymphoproliferative disorders following dermal administration of

ASM 981. 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 ($AUC_{0-24hr} = 643$ and 675 ng·hg/ml for males and females, respectively) for mice after 13 weeks of topical administration of ASM 981 dissolved in ethanol.

Another 13 week dermal toxicity study in mice was conducted to assess the severity of immunosuppression and the rate of onset of lymphoproliferative disorders following dermal administration of ASM 981 to CD-1 mice. Doses of 25 mg/kg/day and above by dermal administration for 13 weeks were associated with lymphoproliferative changes indicative of immunosuppression, 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.

A 52 week dermal carcinogenicity study in mice was conducted with a high dose group that 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. 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. The results from both of these studies are summarized under the carcinogenicity studies section.

Two 4-week oral reproductive hormone studies were conducted in male and female Wistar rats. Doses of 0, 10 and 40 mg/kg/day ASM 981 were administered in both studies. The purpose of these two oral toxicity studies was to investigate a histopathological indication of reproductive hormone suppression and its possible relationship to thymoma formation noted in the oral rat carcinogenicity study. Common findings indicative of immunosuppression noted in other oral toxicity studies conducted in rats were also observed in both studies. The results from the study conducted in male rats suggested that moderate suppression of testosterone secretion might 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.

Estrogen levels were decreased in high dose female 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 from the female rat study, 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 secretion. 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 play a factor in the thymoma formation noted in the oral carcinogenicity study in Wistar rats. It is my belief that the thymoma noted in the oral rat carcinogenicity study is probably more related to systemic immunosuppression associated with ASM 981 administration since the thymus has been characterized as a target organ in repeat dose oral toxicity studies conducted in Wistar rats. The results from the oral carcinogenicity study are summarized in the carcinogenicity study section of this review.

Doses of 0, 1, 5 and 25 mg/kg/day ASM 981 were administered in the 26 week oral study in rats. 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 ($AUC_{0-24 \text{ hr}} = 17.5$ and 23.7 ng·hr/ml for males and females, respectively) for rats after 26 weeks of oral administration of ASM 981 (solid dispersion).

Doses of 0 (untreated control), 0 (vehicle control; 0%), 2 (0.2%), 6 (0.6%) and 10 (1.0%) mg/kg/day ASM 981 were administered in the 26 week dermal study in rats. 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; $AUC_{0-24 \text{ hr}} = 4.9$ 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 $AUC_{0-24 \text{ hr}}$ 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 $AUC_{0-24 \text{ hr}}$ values that were greater than the 26 week oral rat NOAEL. Therefore, no systemic toxicity would be anticipated in this study. However, the design of the study was 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.

Doses of 0, 5, 15 and 45 mg/kg/day ASM 981 were administered in the 4 week oral study in juvenile minipigs. Potential target organs of toxicity identified in this study included the adrenals, thymus and small arteries in minipigs. The effects noted in the adrenals and 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. This effect was noted in the 2 week repeat dose oral toxicity study in adult minipigs but not in a 4 week repeat dose oral toxicity study in adult minipigs. The sponsor argues that the effects in small arteries is a spontaneous lesion in this species of minipigs. ASM 981 appears to cause an increase in the incidence and/or severity of this lesion. This becomes more clear in the 26 week oral study in adult minipigs described next. It would appear that the toxicity associated with oral ASM 981 is similar in juvenile and adult minipigs. The NOAEL identified in this study was 5 mg/kg/day ($AUC_{0-24 \text{ hr}} = 545$ and 444 ng·hr/ml for males and females, respectively) for juvenile minipigs after 4 weeks of oral administration of ASM 981 (solid dispersion).

Doses of 0, 2, 8 and 30 mg/kg/day ASM 981 were administered in the 26 week oral study in minipigs. Potential target organs of toxicity identified in this study included the arteries, adrenals and lungs in minipigs. Arteries were considered the major target organ for toxicity in minipigs. Damage to the arteries in the adrenals was noted in all high dose animals and in various other organs in some of the high dose animals. Effects in adrenals and lungs were noted in mid and high dose animals. The effects noted in the adrenals and lungs were probably related to the exaggerated pharmacology of ASM 981. It is stated in the study report that the arteritis could be caused by a bacterial infection spread in the vascular system as a sequela due to immunosuppression. It would appear that arteritis became more prominent in minipigs after treatment with ASM 981 for a longer duration of treatment. The NOAEL identified in this study was 2 mg/kg/day ($AUC_{0-24 \text{ hr}} = 316$ and 305 ng·hr/ml for males and females, respectively) for minipigs after 26 weeks of oral administration of ASM 981 (solid dispersion).

Doses of 0 (untreated control), 0 (vehicle control; 0%), 4 (0.2%), 12 (0.6%) and 20 (1.0%) mg/kg/day ASM 981 were administered in a 13 week dermal study in juvenile minipigs. No systemic or local dermal toxicity was noted in this study. Therefore, the NOAEL identified in this study was 20 mg/kg/day (1% ASM 981 cream) for juvenile minipigs after 13 weeks of topical administration of ASM 981 cream. It is unclear why no consistent systemic absorption was noted in juvenile minipigs after 13 weeks of repeat dose administration of ASM 981 cream but was noted after 4 weeks of repeat dose topical exposure to ASM 981 cream in minipigs.

It is not surprising that no systemic toxicity was noted in this study since basically no systemic exposure or very low levels of systemic exposure was noted in this study. 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.

Doses of 0 (untreated control), 0 (vehicle control; 0%), 4 (0.2%), 12 (0.6%) and 20 (1.0%) mg/kg/day ASM 981 were administered in a 26 week dermal study in adult minipigs. No systemic or local dermal toxicity was noted in this study. Therefore, the NOAEL identified in this study was 20 mg/kg/day (1% ASM 981 cream; $AUC_{0-24 \text{ hr}} = 7.2$ and 2.8 ng-hr/ml for males and females, respectively) for minipigs 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 minipigs was 2 mg/kg/day. The $AUC_{0-24 \text{ hr}}$ values for this dose were 316 and 305 ng-hr/ml for males and females, respectively. The high dose group in this 26 week minipig dermal toxicity study did not yield $AUC_{0-24 \text{ hr}}$ values that were greater than the 26 week oral minipig 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.

A brief tabular summary of toxicities after long term ASM 981 treatment with associated NOAEL doses and corresponding AUC levels across species is provided in the following table.

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Species	Duration/ Route	Dose (mg/kg/day)	Major findings
Mouse	13 weeks/ oral	0, 10, 50, 100, 312.5	NOAEL = 10 mg/kg/day; $AUC_{0.5-4hr} = 1029$ and 2949 ng-hg/ml in males and females, respectively ≥ 50 mg/kg/day: reduced lymphocyte count, cortical hyperplasia in thymic cortex, medullary thymic atrophy, pleomorphic lymphoid proliferation in spleen ≥ 100 mg/kg/day: reduced serum magnesium level, one lymphoma in spleen and mesenteric lymph nodes (female) 312.5 mg/kg/day: one lymphoma in thymus, islet cell vacuolation, uterine atrophy, vaginal epithelial hypertrophy (female)
Mouse (special studies)	13 weeks/ dermal (ethanol)	Study 1: 0.1 – 50 Study 2: 25 – 200	Study 1: NOAEL for lymphoproliferative changes = 10 mg/kg/day; $AUC_{0-24hr} = 643$ and 675 ng-hg/ml for males and females, respectively ≥ 25 mg/kg/day: lymphoproliferative changes, including malignancies Study 2: No NOAEL for lymphoproliferative changes was established in this study ≥ 25 mg/kg/day: lymphoproliferative changes, including malignancies ≥ 100 mg/kg/day: pleomorphic lymphoma noted after 8 weeks of treatment
Rats	26 weeks/ oral	0, 1, 5, 25	NOAEL = 1 mg/kg/day; $AUC_{0-24 hr} = 17.5$ and 23.7 ng-hr/ml for males and females, respectively ≥ 5 mg/kg/day: medullary thymic atrophy, reduced lymphocyte count, reduced serum magnesium level 25 mg/kg/day: reduced body weight, inflammatory lung lesions, islet cell vacuolation, reduced pancreas weight (female), tubular basophilia and mineralization of corticomedullary junction in kidney, thickening of urinary bladder, increased serum urea and urine volume, bone marrow atrophy, depletion of trabecular and cortical bone, lower pituitary and prostate weight, lens cataracts
Rats	26 weeks/ dermal (FMF)	0 (0%), 2 (0.2%), 6 (0.6%), 10 (1.0%)	NOAEL = 10 mg/kg/day (1% ASM 981 cream); $AUC_{0-24 hr} = 4.9$ ng-hr/ml for males and females Slight thickening of epidermis in control and 10 mg/kg/day groups. No systemic toxicity noted.
Minipigs (juvenile)	4 weeks/ oral	0, 5, 15, 45	NOAEL = 5 mg/kg/day; $AUC_{0-24 hr} = 545$ and 444 ng-hr/ml for males and females, respectively ≥ 15 mg/kg/day: lymphoid cell accumulation in adrenals

			45 mg/kg/day: depression, diarrhea, reduced food consumption, decreased body weight, decreased serum phosphorus, calcium, magnesium and albumin, decreased thymus weight, thymus atrophy, minimal necrotizing arteritis (males)
Minipig	26 weeks/ oral	0, 2, 8, 30	NOAEL = 2 mg/kg/day; AUC _{0-24 hr} = 316 and 305 ng-hr/ml for males and females, respectively ≥ 8 mg/kg/day: accumulation of lymphocytes in adrenals, increased bronchi associated lymphoid tissue, decreased serum phosphorus and magnesium 30 mg/kg/day: mortality due to arteritis, decreased body weight gain, increased adrenal weight, decreased thymus weight
Minipig (juvenile)	13 weeks/ dermal (FMF)	0 (0%), 4 (0.2%), 12 (0.6%), 20 (1.0%)	NOAEL = 20 mg/kg/day (1% ASM 981 cream); no AUC could be determined in this study No systemic or local dermal toxicity was noted in this study.
Minipig	26 weeks/ dermal (FMF)	0 (0%), 4 (0.2%), 12 (0.6%), 20 (1.0%)	NOAEL = 20 mg/kg/day (1% ASM 981 cream); AUC _{0-24 hr} = 7.2 and 2.8 ng-hr/ml for males and females, respectively No systemic or local dermal toxicity was noted in this study.

Toxicology conclusions:

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

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GENETIC TOXICOLOGY:**Genetic Toxicology Study #1:**

Mutagenicity test using Salmonella typhimurium

Study Title: Mutagenicity test using *Salmonella typhimurium*

Key Findings: ASM 981 was negative in the Ames test.

Study No: T-119/203-018
Laboratory Study No: Mut.Bakt. 34/94
Study Type: Ames test
Volume # and Page #: 74, 5-1
Conducting Laboratory: Sandoz Pharma Ltd., Basle, Switzerland
Date of Study Initiation: 7/29/94
GLP Compliance: Yes
QA- Reports: Yes (X) No ()
Drug Lot Number: ASM 981 – Batch# 94901
Formulation/vehicle: DMSO

Methodology:

- Strains: *Salmonella typhimurium* strains TA97a, TA98, TA100, TA102 and TA1535
- Dose Selection Criteria:
 - Basis of dose selection: Toxicity
 - Range finding studies: Performed in all tester strains. No toxicity was noted up to 5000 µg/plate (the highest concentration tested).
 - Test Agent Stability: Slight precipitation was noted at the 1000 µg/plate concentration. More precipitation was noted at the 5000 µg/plate concentration. Chemical analysis of dosing solutions used in the assay demonstrated a purity of — by HPLC. ASM 981 dosing solutions were stable over the dose range tested in this assay.
- Metabolic Activation System: Aroclor 1254 induced rat liver S-9 plus cofactors
- Controls:
 - Vehicle: DMSO
 - Negative Controls: N/A
 - Positive Controls:
 - 1) TA 97a strain – 9-Aminoacridine (-S9, 100 µg/plate) and 2-Aminoanthracene (+S9, 3 µg/plate)
 - 2) TA 98 strain – 2-Nitrofluorene (-S9, 2 µg/plate), 2-Aminoanthracene (+S9, 3 µg/plate) and Benzo(a)pyrene (+S9, 3 µg/plate)
 - 3) TA 100 strain – N-Methyl-N'-nitro-N-nitrosoguanidine (-S9, 3 µg/plate) and 2-Aminoanthracene (+S9, 3 µg/plate)
 - 4) TA 102 strain – Mitomycin C (-S9, 0.5 µg/plate) and 2-Aminoanthracene (+S9, 3 µg/plate)

5) TA 1535 strain – N-Methyl-N'-nitro-N-nitrosoguanidine (-S9, 3 µg/plate) and 2-Aminoanthracene (+S9, 3 µg/plate)

- Comments: Appropriate positive controls were used for each bacterial strain.

- Exposure Conditions:

- Incubation and sampling times: After all treatments had been performed, plates were incubated at $37 \pm 1^\circ\text{C}$ for 48 - 72 hrs. Plates were counted for colony formation after completion of the incubation period.
- Doses used in definitive study: 0, 8, 40, 200, 1000 and 5000 µg/plate (duplicate sets run for each strain)
- Study design: Followed ICH protocol

- Analysis:

- No. plates analyzed: 3 plates/dose
- Counting method: automatic colony counter

- Criteria for Positive Results: A test compound was judged to be mutagenic in this assay if it produces, in at least one concentration and one strain, a response equal to twice (or more) the control incidence. The only exception is for strain TA102 that has a spontaneous revertant number of more than 200. For this strain an increase by a factor of 1.5 over the control level was taken as an indication of a mutagenic effect.

Summary of individual study findings:

- Study Validity: Solvent control mean reversion frequencies fell within established ranges. Positive control results were appropriate showing a mean reversion frequency that was three times or more greater than the mean reversion frequency of the solvent control plates. Dose range selected for the definitive study was appropriate according to ICH guidelines.

- Study Outcome: The test article produced a negative response in the presence and absence of S-9 activation. All of the tester strains treated with the test article exhibited a mean reversion frequency that was similar to the corresponding solvent control.

Genetic Toxicology Study #2:

Mutagenicity test using Salmonella typhimurium

Study Title: Mutagenicity test using *Salmonella typhimurium*

Key Findings: ASM 981 was negative in the Ames test.

Study No: T-120/203-215

Laboratory Study No: 981681

Study Type: Ames test

Volume # and Page #: 74, 5-60
Conducting Laboratory: Sandoz Pharma Ltd., Basle, Switzerland
Date of Study Initiation: 2/15/98
GLP Compliance: Yes
QA- Reports: Yes (X) No ()
Drug Lot Number: ASM 981 – Batch# 97901
Formulation/vehicle: DMSO

Methodology:

- Strains: *Salmonella typhimurium* strains TA97a, TA98, TA100, TA102 and TA1535
- Dose Selection Criteria:
 - Basis of dose selection: Toxicity
 - Range finding studies: Performed in all tester strains. No toxicity was noted up to 2000 µg/plate (the highest concentration tested).
 - Test Agent Stability: Slight precipitation was noted at doses ≥ 1000 µg/plate. Chemical analysis of dosing solutions used in the assay demonstrated a purity of — by HPLC. ASM 981 dosing solutions were stable over the dose range tested in this assay.
- Metabolic Activation System: Aroclor 1254 induced rat liver S-9 plus cofactors
- Controls:
 - Vehicle: DMSO
 - Negative Controls: N/A
 - Positive Controls:
 - 6) TA 97a strain – 9-Aminoacridine (-S9, 100 µg/plate) and 2-Aminoanthracene (+S9, 10 µg/plate)
 - 7) TA 98 strain – 2-Nitrofluorene (-S9, 2 µg/plate), 2-Aminoanthracene (+S9, 3 µg/plate) and Benzo(a)pyrene (+S9, 3 µg/plate)
 - 8) TA 100 strain – Sodium azide (-S9, 3 µg/plate) and 2-Aminoanthracene (+S9, 3 µg/plate)
 - 9) TA 102 strain – Mitomycin C (-S9, 0.5 µg/plate) and 2-Aminoanthracene (+S9, 10 µg/plate)
 - 10) TA 1535 strain – Sodium azide (-S9, 3 µg/plate) and 2-Aminoanthracene (+S9, 3 µg/plate)
- Comments: Appropriate positive controls were used for each bacterial strain.
- Exposure Conditions:
 - Incubation and sampling times: After all treatments had been performed, plates were incubated at 37 ± 1°C for 48 - 72 hrs. Plates were counted for colony formation after completion of the incubation period.
 - Doses used in definitive study: 0, 125, 250, 500, 1000 and 2000 µg/plate (duplicate sets run for each strain)
 - Study design: Followed ICH protocol
- Analysis:

- No. plates analyzed: 3 plates/dose
- Counting method: automatic colony counter

- Criteria for Positive Results: A test compound was judged to be mutagenic in this assay if it produces, in at least one concentration and one strain, a response equal to twice (or more) the control incidence. The only exception is for strain TA102 that has a spontaneous revertant number of more than 200. For this strain an increase by a factor of 1.5 over the control level was taken as an indication of a mutagenic effect.

Summary of individual study findings:

- Study Validity: Solvent control mean reversion frequencies fell within established ranges. Positive control results were appropriate showing a mean reversion frequency that was three times or more greater than the mean reversion frequency of the solvent control plates. Dose range selected for the definitive study was appropriate according to ICH guidelines.

- Study Outcome: The test article produced a negative response in the presence and absence of S-9 activation. All of the tester strains treated with the test article exhibited a mean reversion frequency that was similar to the corresponding solvent control.

Genetic Toxicology Study #3:

Mutation assay at the thymidine kinase locus of mouse lymphoma L5178Y cells

Study Title: Mutation assay at the thymidine kinase locus of mouse lymphoma L5178Y cells

Key Findings: ASM 981 was negative in the mutation assay at the thymidine kinase locus of mouse lymphoma L5178Y cells.

Study No: T-121/203-125
Laboratory Study No: ML 2
Study Type: L5178Y/TK+/- mouse lymphoma assay
Volume # and Page #: 47, 5-87
Conducting Laboratory: Sandoz Pharma Ltd., Basle, Switzerland
Date of Study Initiation: 7/95
GLP Compliance: Yes
QA- Reports: Yes (X) No ()
Drug Lot Number: ASM 981 – Batch# 95906
Formulation/vehicle: DMSO

Methodology:

- Cell line: L5178 cells, clone 3.7.2C
- Dose Selection Criteria:
 - Basis of dose selection: Toxicity. Toxicity was measured by assessing cloning efficiency after treatment of the cell suspension with the test article for 3 or 24 hours. Relative survival was calculated by multiplying “cloning efficiency”

- with "cell counts day 0" of the solvent control cultures. Five concentrations of test article are selected for the definitive test that are between 10% - 100% relative survival.
- Range finding studies: Solvent alone and six concentrations of the test article, separated by 2 fold intervals, and ranging from 15.6 – 500 µg/ml and 31.3 – 1000 µg/ml without and with S9, respectively, were used in the range finding studies. Cells were treated for 24 hours without S9 and for 3 hours with S9. Relative survival was 50.4% at the highest dose tested without S9 (500 µg/ml). Relative survival was 63.1% and 39.3% in the presence of S9 at concentrations of 500 and 1000 µg/ml, respectively.
 - Test Agent Stability: ASM 981 precipitated in the treatment medium at concentrations ≥ 231 µg/ml. Chemical analysis of dosing solutions used in the assay demonstrated a purity of — by HPLC. ASM 981 dosing solutions were stable over the dose range tested in this assay.
 - Metabolic Activation System: Aroclor 1254 induced rat liver S-9 plus cofactors
 - Controls:
 - Vehicle: DMSO
 - Negative Controls: N/A
 - Positive Controls: Methyl Methanesulfonate was used in the non-activated system (10 µg/ml for 24 hour exposure). Benzo(a)pyrene was used in the activated system (1.5 µg/ml for 3 hour exposure).
 - Comments: Appropriate positive controls were used in this study.
 - Exposure Conditions:
 - Incubation and sampling times: Cell suspensions were incubated with test article with (3 hr exposure) or without S9 activation (24 hr exposure). Cell suspensions were then cultured either on viable count control plates or trifluorothymidine plates (the selective agent; 3 µg/ml TFT). The plates were incubated at $37 \pm 1^\circ\text{C}$ for 12 days. Plates were counted for large and small colony formation after completion of the incubation period.
 - Doses used in definitive study:
First experiment: 0, 10, 30, 100, 300 and 1000 µg/ml without S9; 0, 50, 100, 200, 400 and 800 µg/ml with S9
Second experiment: 0, 15, 30, 60, 180 and 300 µg/ml without S9; 0, 116, 231, 278, 333 and 400 µg/ml with S9
 - Study design: Followed ICH protocol
 - Analysis:
 - No. replicates: 2/dose
 - Counting method: automatic colony counter
 - Criteria for Positive Results: The test article was considered positive if one or more of the mutant frequencies in the treated groups was statistically significantly ($p < 0.05$) larger than the corresponding solvent control value and there was a significant ($p < 0.05$) dose relationship indicated by linear trend.

Summary of individual study findings:

- Study Validity: Solvent control mutant frequencies fell within established ranges. Positive control results were appropriate. Dose range selected for the definitive study was appropriate according to ICH guidelines.
- Study Outcome: The test article produced a negative response in the presence and absence of S-9 activation. All of the concentrations tested in this study exhibited a mutant frequency that was similar to the corresponding solvent control.

Genetic Toxicology Study #4:*Chromosomal aberration test with V79 Chinese hamster cells*

Study Title: Chromosomal aberration test with V79 Chinese hamster cells

Key Findings: ASM 981 was negative in the chromosomal aberration test in Chinese hamster cells.

Study No: T-123/203-021
Laboratory Study No: Z52
Study Type: Chromosomal aberration assay
Volume # and Page #: 47, 5-177
Conducting Laboratory: Sandoz Pharma Ltd., Basle, Switzerland
Date of Study Initiation: 10/3/94
GLP Compliance: Yes
QA- Reports: Yes (X) No ()
Drug Lot Number: ASM 981 – Batch# 94902
Formulation/vehicle: DMSO

Methodology:

- Cell line: V9 Chinese hamster cells
- Dose Selection Criteria:
 - Basis of dose selection: Toxicity and morphology changes. Toxicity was assessed in treated cells by the % reduction in cell growth in comparison to control. In addition, treated plates were evaluated for effects of treatment on the morphology of the cells. A % reduction in cell growth <75% was considered significant.
 - Range finding studies: Seeded plates were treated with either solvent alone or various concentrations of the test article ranging from 10 – 1000 µg/ml. Two tests were run with 3 hr incubations with test article (-S9 and +S9) and one test was run with a 20 hr incubation (-S9). Changes in cell morphology were noted at concentrations > 492 µg/ml after 3 hr treatment and > 119 µg/ml after 20 hr treatment in the absence of S9. Cell growth was reduced at concentrations ≥ 20 µg/ml after 3 hr treatment and at ≥ 10 µg/ml after 20 hr treatment in the absence of S9. Changes in cell morphology were noted at