Summary: Intravenous infusion of levo-ASL-8052 or dl-ASL-8052 over a 1 hour interval at increasing concentrations in 4 dogs indicates that 120 mg/kg (2000 mcg/kg/min) is the maximum tolerated dose and that 180 mg/kg (3000 mcg/kg/min) can produce salivation, tremors, ataxia, sedation, hyperpnea and death. Terminating the infusion of high doses (240 mg/kg, 4000 mcg/kg/min) prevented death in about half of the animal. In animal which survived, recovery occurred within 5 min.

(8) Acute i.v. Toxicity of a 10% Propylene Glycol / 10% Alcohol Formulation of ASL-8052 in Rats: Separate groups of 10 rats (Charles River CD)/sex/dose received a single dose of ASL-8052 (ranging from 50 to 90 mg/kg) intravenously (tail vein). Mortality, signs of toxicity, and body weight were recorded over a 14 day observation period. Each dose was administered in a volume of 10 ml/kg at a rate of 2 ml/min (controlled by a syringe pump). The vehicle for ASL-8052 contained sodium acetate, glacial acetic acid, 10% propylene glycol, 10% alcohol and water. NaOH and HCl were used to titrate pH to 5.0. One rat dosed with ASL-vehicle showed sedation otherwise there were no mortalities or effects seen with either the vehicle of saline.

	MALE	FEMALE
Doses Tested, mg/kg	50, 60, 75, 90	
LD50, mg/kg (95% C.L.)	61.0 (57.3-68.8)	71.7 (65.3-78.7)
Combined LD50, mg/kg (95% C.L.)	66.7 (62.8-70.8)	
Highest Non-Lethal Dose, mg/kg	50.0	50.0
Lowest Lethal Dose, gm/kg (#dead/#tested)	60.0(4/10)	60.0(1/10)
Total Deaths/# Given Lethal Dose	24/30	17/30
Day of Deaths	Day 1: 24/24	17/17

All deaths occurred within 5 min of dosing. There were no surviving males in the 75 and 90 mg/kg dose groups and only 1 surviving female in the 90 mg/kg group.

Signs of Toxicity were seen at all doses. Effects seen in more tha 20% of the ASL-treated animals included prostration (65%), ataxia (45%), dyspnea (28%), and sedation (24%). Effects observed in less than 20% of the animals included tonic convulsions, hypopnea, tremors, and hyperpnea. All signs of toxicity disappearance within 8 min of administration. Weight gains of surviving rats were essentially the same in all groups. There was no remarkable drug-related pathology at necropsy.

NOTE: The Sponsor does not indicate signs of toxicity in animals which died. The most common sign at doses which did not cause mortalities was sedation. Effects seen only in dose-groups in which mortalities occurred included dyspnea, tonic convulsions, and hyperpnea. These groups also had the highest incidence of prostration and ataxia.

Summary: In rats, the i.v. administration of 50 mg/kg ASL-8052 in an acetate/acetic acid/10% propylene glycol/10% alcohol/water formulation caused sedation, prostration, ataxia and hypopnea but was not lethal. These effects lasted less than 8 minutes. Doses of 60 mg/kg or greater caused lethalities, all of which occurred within 5 minutes. The LD50 of ASL-8052 in the present formulation (M: 61.0 mg/kg; F: 71.7 mg/kg; combined: 66.7 mg/kg) was essentially the same as that obtained in a previous study using saline as the vehicle (M: 69.8 mg/kg; F: 71.5 mg/kg: Combined: 70.9 mg/kg).

(9) Comparison of the Acute i.v. Toxicity of Two ASL-8052 Formulations (10% Propylene Glycol / 10% Alcohol and 20% Glycerin / 60% Alcohol) in Rats: Separate groups of 10 rats (CD)/sex/dose received a single dose of either:

1) 0.9% Sodium Chloride Injection, USP;
2) Vehicle (lot No. 899-32) (20% Glycerin USP, 60% Alcohol USP, Water for Injection USP) diluted with 0.9% Sodium Chloride Injection USP to the highest concentration of 25% ASL-8052 formulation used in this study;
3) ASL-8052 25% Formulation (lot No. 899-26) (ASL-8052 250 mg/ml in above vehicle) appropriately diluted with Sodium Chloride Injection USP; or
4) ASL-8052 10% Formulation (lot No. 802-31) (100 mg/ml ASL-8052 in 10% Propylene Glycol USP, 10% Alcohol USP Water for Injection USP) appropriately diluted with 0.9% Sodium Chloride Injection USP.

The two ASL-8052 formulations were diluted to equal concentrations of 5, 6, 7.5 and 9.0 mg/ml and infused i.v. (tail vein). Each dose was administered in a volume of 10 ml/kg at a rate of 2.0 ml/min (controlled by a syringe pump). Mortality, signs of toxicity, and body weight were recorded over a 14 day observation period.

	MALE	FEMALE
10% ASL-8052		
Doses Tested, mg/kg	50, 60, 75, 90	
LD50, mg/kg (95% C.L.)	54.1 (43.5-60.0)	64.6 (58.5-71.1)
Combined LD50, mg/kg (95% C.L.)	59.1 (54.4-63.3)	
Highest Non-Lethal Dose, mg/kg	0	50
Lowest Lethal Dose, gm/kg (#dead/#tested)	50 (4/10)	60 (5/10)
Total Deaths/# Given Lethal Doses	30/40	22/30
Day of Deaths	Day 1: 30/30	22/22
	All deaths occurred within 10 min of dosing. There were no surviving males in the 75 and 90 mg/kg groups, respectively and 3 and 0 surviving females in the 75 and 90 mg/kg group, respectively.	
25%-ASL-8052		
Doses Tested, mg/kg	50, 60, 75, 90	
LD50, mg/kg (95% C.L.)	57.2 (45.7-64.7)	63.8 (52.1-74.5)
Combined LD50, mg/kg (95% C.L.)	60.3 (53.3-66.0)	
Highest Non-Lethal Dose, mg/kg	0	0
Lowest Lethal Dose, gm/kg (#dead/#tested)	50 (2/10)	50 (2/10)
Total Deaths/# Given Lethal Doses	27/40	22/40
Day of Deaths	Day 1: 27/27	22/22
	All deaths occurred within 10 min of dosing. There were 1 surviving male in each the 75 and 90 mg/kg groups. There were 4 and 1 surviving females in the 75 and 90 mg/kg groups, respectively.	

Signs of Toxicity were seen at all doses and were dose-related. The most common signs seen with both compounds included prostration, ataxia and dyspnea. Sedation, tremors and clonic convulsions were only occasionally seen. All toxic signs disappearance within 10 minutes. There was no notable drug-related difference in weight gains of survivors. There were no clear differences between the 10% and 25% ASL-8052 Formulations even though the latter contained much more alcohol than the latter.

NOTE: The Sponsor did not indicate signs of toxicity in animals which died. The most common signs at the two highest doses were prostration, ataxia and dyspnea.

Summary: In rats, the acute i.v. toxicity (based on LD_{50}'s and signs of toxicity) was essentially the same for a 10% ASL-8052 formulation in 10% propylene glycol and 10% alcohol as it was for a 25% ASL-8052 formulation in 20% glycine and 60% alcohol. The acute toxicities of these formulations were also similar to that of ASL-8052 raw material.

b. ACUTE ORAL TOXICITY:

Separate groups of 10 rats (Charles River CD)/sex/dose received a single dose of ASL-8052 (ranging from 1.0 to 15 gm/kg) by gavage. Mortality, signs of toxicity, and body weight were recorded over a 14 day observation period. Separate LD50's for male and female rats were not computable by prohibit analysis.

		MALE	FEMALE
Doses Tested, gm/kg		1, 2, 4, 8, 11 &15	
Combined LD50, gm/kg (95% C.L.)		8.9 (8.29-9.62)	
Highest Non-Lethal Dose, gm/kg:		4	8
Lowest Lethal Dose, gm/kg (#dead/#tested)		8 (4/10)	11 (9/10)
Total Deaths/# Given Lethal Dose		24/30	19/20
Day of Deaths	Day 1:	20/24	10/19
	Day 2:	4/24	9/19

Signs of Toxicity: None at 1 gm/kg in Males and 1 or 2 gm/kg in females. Sedation at 2 gm/kg and above. Prostration at 8 gm/kg and above. Tremors, dyspnea, hypokinesia, ataxia, clonic convulsions and cool to touch were seen at 11 and 15 gm/kg. Sponsor does not indicate which signs proceed deaths but the signs seen at 8 gm/kg and above were only seen in groups in which deaths occurred. No toxic signs were seen after 2 days. There were no differences in body weights or body weight gains in surviving rats receiving up to 8 gm/kg, however, these measures were clearly reduced in the 1 rat (F) which survived 11 gm/kg. There were no clearly drug related findings at necropsy.

Summary: In rats, single oral doses of ASL-8052 caused no observable effects at up to 1 gm/kg and no deaths at up to 4 gm/kg. The only effect observed at non-lethal doses was sedation. Prostration was observed at 8 gm/kg and above. Tremors, dyspnea, hypokinesia, ataxi, clonic convulsions and cool to touch were seen at 11 & 15 gm/kg. Aigns of toxicity were seen for up to 2 days. The oral LD_{50} (8.91 gm/kg) was approximately 125 times grater than the i.v. LD_{50} (70.9 mg/kg) in rats. Most of the deaths occurred on the day of dosing and the remainder occurred on the following day. These results suggest that oral ASL-8052 is poorly absorbed and/or rapidly metabolized.

3. Sub-Chronic Toxicity: The sponsor submitted an in-house 7 day i.v. dose-ranging study (DR-study) in the rat, a 5 day i.v. dose ranging study (DR-study) in the dog performed in-house, and 2-week i.v. studies (2-wk studies) in the rat and dog performed by IRDC, Mattawan, MI. Blood levels of ASL-8052 and its acid metabolite, ASL-8123 were measured by the Sponsor in blood samples from the in-house DR-study in the dog and in samples from2-wk studies in the rat and dog performed by IRDC. The vehicle used for these studies was 90% sodium chloride for injection, 7% propylene glycol USP, 2% alcohol USP, and water for injection USP.

a. Rat. Both the DR-study (5/sex/dose level) and the 2 wk-study (15/sex/dose level) used Charles River CD rats of comparable size (DR study: m/209-295g & F/148-196g; 2 wk study: M/127-239g & F/133-250g). ASL-8052 was infused i.v. (tail vein) at the appropriate drug concentrations (supplied by Sponsor & calculated so that each dose was given in a volume of 10 ml/kg) once a day for either 7 or 14 consecutive days at a rate of 4 ml/min. Doses tested included 12, 18, 27, 40 or 60 mg/kg/day in the initial DR-study and 5, 20 or 40 mg/kg/day in the 2 wk-study. In both studies separate groups of animals received vehicle.

Deaths occurred only at daily doses of 40 mg/kg (DR: 3/10 & 2 week: 4/30) and above (9/10 at 60 mg/kg/day). All deaths occurred within minutes of dosing; 8 of the 16 rats died on DAy 1 while 5 rats died on Day 2 (1 at 40 mg/kg and 4 at 60 mg/kg) and 3 died on day 4 (2 at 40 mg/kg and 1 at 60 mg/kg).

The 5 mg/kg/day X 14 days caused no observable effects while 12 mg/kg day and 18 mg/kg/day caused reduced motor activity (1-3 rats) and at the higher dose sedation (1 rat). The incidence and types of behavioral effects were clearly dose related. Thus, effects seen at 20 mg/kg/day in several rats not only included effects seen at the lower doses but also included respiratory depression and ataxia. Similar effects were also seen at 27 mg/kg/day but in a larger proportion of the animals. Lethal doses (40 & 60 mg/kg/day) in addition to the effects described above also caused dyspnea & prostration in many of the animals and hyperpnea, blanched eyes, unconsciousness, tremors and convulsions in a few animals. Surviving animals appeared normal in less than 30 minutes. There were no drug related effects on body weight gains or food consumption or on terminal (overnight fast) hemotalogy (RBC, Hct, Hgb, MCH, MCV, MCHC, WBC, differential, platelet count or reticulocyte count), serum chemistry (BUN, creatinine, AP, total bilirubin, SGOT, SGPT, LDH, protein, Albumin, A/G ratio, glucose, CPK, PP, APTT, NA^+, K^+, Cl^-, Ca^{++}) or urinalysis (color, appearance microscopic-sediment, volume, pH, glucose, occult blood, nitrites, urobilinogen, ketone & Bilirubin). Pre-and Post-treatment ophthalmoscopic examination failed to reveal adverse effects on the eye at up to 40 mg/kg/day X 2 weeks. At necropsy, except for a decrease in absolute and relative ovarian weight at 20 mg/kg/day, there were no drug related gross or histopathologic changes at up to 40 mg/kg/day X 14 days or 60 mg/kg/day X 7 days in either survivors or nonsurvivors.

Blood samples obtained by IRDC prior to study initiation and again within 1 minute of dosing on Days 1, 2, 6, 7 & 14 were analyzed for levels of ASL-8052 and ASL-8123 by the sponsor. Blood levels of ASL-8052 (1.45, 5.64 & 14.6 mcg/ml) and ASL-8123 (5, 11.3, 18.9 mcg/ml) were directly related to dose (5, 20 and 40 mg/kg/day, respectively). There were no sex differences in plasma levels or accumulation of either compound over the 14 day dosing period. The t 1/2 was less than 1 min., probably because of rapid hydrolysis in blood and tissues. The decrease of the ASL-8123/ASL-8025 ratio (3.45 to 1.29) from the low to the high dose suggests saturation of the enzyme system.

b. Dog: Beagle dogs 7-8.5 months of age were used in both the DR-study (1/sex/dose level) and the 2 wk study (3/sex/dose level). ASL-8052 was continuously (24 hrs/day) infused i.v. (commulated jugular vein) at 0.4 ml/kg/min at appropriate concentrations supplied by the sponsor. Concentrations were calculated so that rates of drug administration in the initial DR-study were 50, 100, 200, 500 or 1000 mcg/kg/min 24 hrs/day for 5 days and 100, 400 or 800 mcg/kg/min 24 hrs/day in the 2 wk study. In both studies separate groups of animals received vehicle and in the 2 wk study one group of dogs received saline alone.

There were no mortalities at up to 1000 mcg/kg/min 24 hours/day for 5 days or up to 800 mcg/kg/min 24 hours/day for 14 days.

In a pilot study ASL-8052 was infused at 1000 mcg/kg/min in 1 male and 1 female dog. The male dog became ataxic after about 20 hours of infusion and, when the infusion rate was increased to 2000 mcg/kg/min, the dog had severe convulsions tremors and muscular rigidity after about 22 hours. The female exhibited tonic convulsions after about 48 hours of infusion at 1000 mcg/kg/min. Therefore, lower doses were tested in the DR-study and the 2 wk study. Although sedation and diarrhea were reported in 1 out of 8 dogs at 100 mcg/kg/min no effects were reported at 50, 200 or 400 mcg/kg/min. Effect observed at 500 mcg/kg included hyperexcitability in one male and head tremors and muscle rigidity in one female, while 800 mcg/kg/min for 2 wk caused several occasions of emesis (2/6 dogs) and decreased activity (2/8 dog) and in one of 6 dogs prostration, disorientation, ataxia, salivation and decreased muscle tone. An infusion rate of 1000 mcg/kg/min in the DR study caused sedation, head tremors, muscle rigidity, ataxia, salivation, emesis and disorientation in both dogs and clonic convulsions, vocalization, prostration, unconsciousness and ptosis in the female dog.

All groups lost weight equally (up to 7%) during the study. There was a dose related decrease in food consumption, ranging from 10% to 18%, in males in the 2 wk-study. Interestingly, saline controls consumed 20-30% more food than did vehicle controls.

ECG (6 lead with dog lying on right side) recorded before treatment initiation and on Days 7 & 14 of treatment were unremarkable except for catheter induced extrasystoles in all groups (ECG read by D.K. Detweiler, VMD, Univ. Pennsylvania Sch. of Med., Philadelphia, PA).

Ophthalmoscopic examination (indirect opthalmoscope & direct or slit lamp) performed before treatment initiation and on Day 12 failed to suggest and drug effects on the eye at up to 800 mcg/kg/min for 24 hrs/day X 12 days.

Blood samples for hematologic exam (Hgb, Hct, RBC, WBC, MCV, MCH, MCHE, differential, platelets & reticulocytes), and clinical chemistries (BUN, creatinine, AP, glucose, SGOT, SGPT, LDH, CPK, T. bilirubin, T. protein, albumin, A/g ratio, Na^+, K^+, Cl^- Ca^{++} erythrocyte & plasma cholinesterase, prothrombin time & APTT) and urine for urinalysis (color, appearance, microscopic exam, volume, osmolarity, pH, protein, glucose, occult blood, nitrites, urobilinogen, ketone, and bilirubin) were collected after an overnight fast before treatment initiation and again on Day 5 in the DR-study or Days 8 & 13 in the 2wk study. The only statistically significant differences in hematologic values between drug and vehicle treatment animals were seen in females during the 2 wk-study; on day 8 Hgb was lower in the 400 & 800 mcg/kg/min groups and at termination Hgb and Hct were lower in all drug treated groups (100, 400 or 800 mcg/kg/min) and RBC count was lower in the two highest infusion groups. These effects were dose related but within normal limits. Similar differences in male were not unremarkable except for a slight elevation of BUN in high dose females at termination, however this value was not statistically significant compared to vehicle controls. Urinalysis was unremarkable except for elevated proteins. Subsequent studies indicate this result to be a false positive due to the presence of ASL-8052 and/or ASL-8123.

At necropsy there was no drug related gross pathology. Catheter-induced cardiac damage (endocardial) was grossly visible and confirmed histologically in all groups, including controls. Organ weights (thymus, heart, thyroid, brain, pituitary, gonads, spleen, liver, adrenals, kidney, prostate and uterus) were unremarkable except for a slightly lower mean relative liver weight in high dose males. No histopathology was observed in the liver or, except for the heart (see above), in any other organ.

Blood levels of both ASL-8052 and ASL-8123 were determined by the sponsor in blood samples taken before initiation of treatment, 24 hours after initiation of ASL-8052 infusion and on Days 3 & 5 in the DR-study or on Day 4, 7, 13, 14 & 15 in the 2 wk study. Note that methods section for both studies state that blood samples were taken at 1, 5, 10, 15, 30, 45 & 60 minutes after infusion were terminated. However, these data are not included in the toxicology reports or the sponsor generated reports on blood levels.

Results of these studies indicate that 1) there was a dose-related increase in plasma levels of both ASL-8052 & ASL-8123; 2) there was no apparent accumulation of either compound; 3) there was no difference between sexes with regard to plasma levels; and 4) steady state levels of either compound were reached within 24 hours.

In the DR-study there was a disproportionate increase in ASL-8052, but not ASL-8123, levels when the dose was increased from 500 to 1000 mg/kg/min. This was not observed in the 2 wk study when levels at 400 mcg/kg/min were compared to those at 800 mcg/kg/min. In general, blood levels of both compounds were higher in the DR-study than in the 2 wk study. This difference may relate to the small number of animals and/or the use of a different analytical procedure in the DR-study. Nonetheless, at each infusion rate the average steady state level of ASL-8052 was at least 17 times and as much as 113 times the levels of ASL-8052. Using the rate of infusion (mcg/kg/min) and the steady state concentrations at each dose level, the calculated average clearance of ASL-8052 and ASL-8123 were 626 ml/kg/min and 6.27 ml/kg/min respectively. The sponsor points out that at physiologic pH ASL-8123 has a double charge.

Rate of ASL 8052 Infusion (mcg/kg/min)	AVERAGE PLASMA LEVELS, mcg/ml ASL-8052	ASL-8123	Ratio
DR-STUDY			
50	0.534	9.00	16.9
100	0.665	18.3	28.3
200	0.971	45.3	46.7
500	2.15	103	47.9
1000	12.5	278	22.2
2 wk study			
100	0.153	13.75	89.9
400	0.595	67.35	113.2
800	1.45	142	97.9

4. Intravenous and Perivascular Irritation:

a. Rabbit:

(i) 70% Propylene Glycol-20% Alcohol Formulation of ASL-8052:

The standard ASL-8052 formulation (100 mg/ml in 70% propylene glycol USP, 20% alcohol USP & 10% water injection USPO caused an intense and extensive inflammatory and necrotizing reaction in the rabbit ear when administered i.v. (over 30 minutes) or perivascularly (0.1 ml) with little or no resolution during a 10 day interval. A 1:10 (10 mg/ml or 1:30 (3.3 mg/ml) dilution with saline results in mild to slight irritation, respectively, with resolution in less than 10 days. Thus, a saline dilution of the ASL-8052 formulation of 1:100 (1 mg/ml) was no more irritating than saline while a 1:30 dilution (3.3 mg/ml) was the threshold level of producing tissue irritation.

(ii) Comparison of Two ASL-8052 Formulations (20% glycerin-60% Alcohol vs. 10% Propylene Glycol-10% Alcohol):

Separate groups of 6 New Zealand White rabbits (1.84-3.07 kg, sex not stated) received i.v. (right ear) and perivascularly (left ear) either:

1) 0.9% Sodium Chloride Injection, USP;
2) Vehicle (lot No. 899-32) (20% glycerin USP, 60% Alcohol USP, Water for Injection USP) diluted 1 part in 1.5 parts 0.9% Sodium Chloride Injection USP;
3) ASL-8052 250 mg/ml formulation (lot No. 899-26) (Vehicle, see above) appropriately diluted with Sodium Chloride Injection USP; or
4) ASL-8052 100 mg/ml formulation (lot No. 802-31) (10% Propylene Glycol USP, 10% Alcohol USP Water for Injection USP) appropriately diluted with 0.9% Sodium Chloride Injection USP.

The two ASL-8052 formulations were diluted to approximately equal concentrations of 5, 10, 20 (23), 50 (59) and 100 (137) mg/ml and infused i.v. at 1.2 (1.4) mg/min (syringe pump). The numbers in ( ) indicate corrected concentration of the ASL-250 mg/ml formulation after analysis indicated a greater than 10% deviation from the calculated concentration.

Venous Irritation: Test solution was infused (syringe pump) for 6 hours through a teflon catheter inserted into the marginal ear vein of the right ear (catheter was removed immediatly after infusion). Saline and vehicle were infused at 0.240 and 0.012 ml/min, respectively: ASL-8052 solutions were infused at 0.24 to 0.012 ml/min at appropriate concentrations to deliver 1.2 mg/min.

Perivascular Irritation: A single bolus dose of 0.1 ml was injected (27 gauge needle) s.c. alongside the marginal ear vein of the left ear.

Rabbits were treated and observed for venous and perivascular irritation at approximately 1 and 24 hours after treatment. After the 24 hour grading period rabbits were euthanized (CO_2) and each ear prepared for histologic evaluation.

Based on macroscopic mean erythema scores (but not mean edema scores), there was venous and perivascular irritation at nearly all concentrations of both ASL-8052 formulations and vehicle that was significantly greater than saline. The Sponsor, however, states "Based upon analysis of data from previous work it has been concluded that the microscopic evaluations are the mose reliable for determining venous and perivascular irritation. The macroscopic scores failed to respond in a concentration-related manner in many instances and often did not agree with the microscopic results."

The microscopic reactions included hemorrhage, edema, inflammatory infiltrate, occluding and nonoccluding thrombi, necrosis, leucocyte pavementing and venous ectasia (only one rabbit from each formulation). There was no microscopic evidence of venous or perivascular irritation with saline but some irritation was observed in the vehicle group and with most concentrations of both formulations (severity was concentration-dependent). There was substantial venous irritation at concentrations as low as 5 mg/ml of both formulations. Using a parallel-line bioassay analysis the 25% formulations caused significantly more venous and perivascular irritation than the 10% formulation. Since maximal irritation scores were seen at the 2-3 highest, but essentially equal, concentrations of both formulations it appears that the more irritating 25% formulations is not due to ASL-8052 but due to Difference in vehicle formulation. The vehicle used in the 10% ASL-8052 formulation was not tested alone. None the less, ASL-8052 clearly added to the irritation of the vehicle.

Summary: Both formulations of ASL-8052 produced substantial venous irritation at concentrations as low as 5 mg/ml (lowest concentration tested). At equal or similar concentrations the 25% formulation produce significantly more venous and perivascular irritation than the 10% formulation suggesting that the glycerin (20%)-alcohol (60%)-water vehicle is more irritating than the propylene glycol (10%)-alcohol (10%)-water vehicle.

(3) Comparison levo-ASL-8052 with dl-ASL-8052 in a 10% Propylene Glycol-10% Alcohol Formulation:

Separate groups of 6 (3/sex) New Zealand White rabbits (1.24-2.36kg) received i.v. (right ear) and perivascularly (left ear) either:

1) 0.9% Sodium Chloride Injection, USP;
2) dl-ASL-8052 100 mg/ml formulation (lot No. 802-31) (Vehicle: 10% Propylene Glycol USP, 10% Alcohol USP and 0.1M Acetate buffer in Water for Injection USP) appropriately diluted with Sodium Chloride Injection USP; or
3) levo-ASL-8052 100 mg/ml formulation (lot No. 899-39A) (Vehicle: same as for dl-ASL-8052, see above) appropriately diluted with Sodium Chloride Injection USP.

The two ASL-8052 formulations were diluted to approximately equal concentrations of 5, 10, 20 (22), 65 (59) and 100 mg/ml and infused i.v. at 1.2 to 1.6 mg/min (syringe pump). The numbers in ( ) indicate corrected concentration of the levo-ASL-8052 formulation after analysis indicated a greater than 10% deviation from the calculated concentration.

Venous Irritation: Test solutions were infused (syringe pump) for 6 hours through a teflon catheter inserted into the marginal ear vein of the right ear (catheter was removed immediatly after infusion). Saline was infused at 0.240 ; ASL-8052 solutions were infused at 0.24 to 0.012 ml/min at appropriate concentrations to deliver 1.2 to 1.6 mg/min.

Perivascular Irritation: A single bolus dose of 0.1 ml was injected (27 gauge needle) s.c. alongside the marginal ear vein of the left ear.

Rabbits were treated and observed for venous and perivascular irritation at approximately 1 and 24 hours after treatment. After the 24 hour grading period rabbits were euthanized (CO_2) and each ear prepared for histologic evaluation.

Based on macroscopic mean erythema scores (but not mean edema scores), there was venous irritation at all except the 5 mg/ml concentrations of both l-and dl-ASL-8052 that was significantly greater than saline. Only the highest 2-3 concentrations of each formulation caused significantly higher mean venous edema scores than saline. Similarily, only the highest 2-3 concentrations of each formulation caused macroscopic perivascular irritation. The Sponsor, however, states "Based upon analysis of data from previous work it has been concluded that the microscopic evaluations are the most reliable for determining venous and perivascular irritation."

There was no microscopic evidence of venous irritation with saline but there was substantial venous irritation at all but the lowest (5 mg/ml delivered at 1.2 mg/min) concentrations of both formulations (severity was concentration-dependent). Using a parallel-line bioassay analysis the levo-ASL-8052 caused significantly less venous irritation than the dl-ASL-8052. It should be noted that this difference is due to difference in irritation at the lower concentrations and not at the 2 highest concentrations. The microscopic reactions included hemorrhage, edema, inflammatory infiltrate, occluding and nonoccluding thrombi, necrosis, leucocyte pavementing. After perivascular administration there was no irritation with saline while irritation was generally seen at concentration of 10 mg/ml and above with both formulations. There was no difference between the isomeric forms in terms of perivascular irritation. The irritation at the perivascular site was less severe than at the venous site. The vehicle used in these ASL-8052 formulations was not tested alone.

Summary: Microscopically, both the l- and dl-forms of ASL-8052 produced significantly more venous and perivascular irritation 24 hours after a 6 hr i.v infusion or a single perivascular injection, respectively, than saline at concentrations or 10 mg/ml and above. There was a suggestion that the l-form was slightly less irritating than the dl-form but this was not conclusive. No venous or perivascular irritation was observed in saline-treated rabbits or those treated with either l- or dl-ASL-8052 at a concentration of 5 mg/ml delivered at 1.2 mg/min.

(4). Comparison of Vehicle (10% Propylene Glycol-10% Alcohol) with and without ASL-8052:

Separate groups of 6 New Zealand White rabbits (1.03-2.44 kg, sex not stated) received i.v. (right ear) and perivascularly (left ear) either:

1) 0.9% Sodium Chloride Injection, USP;
2) Vehicle (lot No. 899-39B) consisting of 10% Propylene Glycol USP, 10% Alcohol USP and 0.1 M Acetate buffer in Water for Injection USP appropriately diluted with 0.9% Sodium Chloride Injection USP;
3) ASL-8052 (lot No. 907-37) dissolved in 0.9% Sodium Chloride Injection USP;or
4) ASL-8052 100 mg/ml formulation (lot No. 767-49) (Vehicle: 10% Propylene Glycol USP, 10% Alcohol USP and 0.1 M Acetate buffer in Water for Injection USP) appropriately diluted with 0.9% Sodium Chloride Injection USP .

Vehicle and ASL-8052 in vehicle were diluted 1:50, 1:20, 1:10 and 1:5. Vehicle was also tested undiluted and the ASL-8052 formulation was also diluted 1:2. These dilutions of the ASL-8052 formulation resulted in concentrations of 2, 5, 10, 20 and 50 mg/ml. Essentially the same concentrations of ASL-8052 in Saline were prepared. All solutions were infused at a rate of 0.1 ml/min resulting in a delivery of ASL-8052 at 0.2 to 5 mg/min.

Venous Irritation: Test solutions were infused (syringe pump) for 6 hours through a teflon catheter inserted into the marginal ear vein of the right ear (catheter was removed immediately after infusion) at a rate of 0.1 ml/min.

Perivascular Irritation: A single bolus dose of 0.1 ml was injected (27 gauge needle) s.c. alongside the marginal ear vein of the left ear.

Rabbits were treated and observed for venous and perivascular irritation at approximately 1 and 24 hours after treatment After the 24 hour grading period rabbits were euthanized (CO_2) and each ear prepared for histologic evaluation.

Based on macroscopic scores of edema and/or erythema there was venous irritation with the undiluted vehicle and with ASL-8052 solutions at concentration of 8 mg/ml and above delivered at 0.8 mg/min and above in either saline or vehicle. Macroscopic evidence of perivascular irritation was seen with undiluted vehicle and with ASL-8052 in saline at 50 mg/ml and in vehicle at 2 mg/ml and above.

The Sponsor, however, states "Based upon analysis of data from previous work it has been concluded that the microscopic evaluations are the most reliable for determining venous and perivascular irritation. The macroscopic scores were not concentration dependent and in many instances did not agree with the microscopic results."

There was no microscopic evidence of irritation in the saline-treated group of with either venous or perivascular administration. The undiluted vehicle (but not dilutions of 1:50, 1:20, 1:10 or 1:5) caused considerable irritation when administered i.v. or perivascularly. Both ASL-8052 preparations produced significant venous irritation at concentrations of 8-10 mg/ml and higher (delivered at 0.8 mg/ml and above). There was no difference between preparations with regard to venous irritation when subjected to Parallel Line Assay. Microscopic observations included perivenous hemorrhage, perivenous edema, perivenous inflammatory infiltrate, occluding and non occluding thrombi, leucocyte pavementing, and necrosis. The degree of irritations seen with perivascular administration was generally less that that seen with venous administration, however, slightly more irritations was produced by the ASL-8052 formulation than by ASL-8052 in saline. Microscopic observations included hemorrhage, edema and inflammatory infiltrate.

Summary: In the rabbit ear, intravenous or perivascular administration of undiluted vehicle (10% Propylene Glycol USP, 10% Alcohol USP and 0.1 M Acetate buffer in Water for Injection USP) caused substantial irritation. This irritation was eliminated when the vehicle was diluted with saline as little as 1:5. When administered i.v., ASL-8052 at concentrations of about 10 mg/ml or greater in either saline or vehicle (1:10 dilution) caused a concentration-dependent irritation (no significant difference between preparations) that ranged from slight to severe. The ASL-8052 formulation, however, tended to produce slightly more irritation than ASL-8052 in saline when administered perivascularly. These results indicate that ASL-8052 itself is the main cause of local vascular irritation produced by intravenous infusion. This irritation is manifested in perivenous hemorrhage, perivenous edema, perivenous inflammatory infiltrate, occluding and non occluding thrombi, leucocyte pavementing, and necrosis.

(5) Effect of Infusion Volume Flow Rate on Venous Irritation Produced by ASL-8052 in a 10% Propylene Glycol-10% Alcohol Formulation:

Separate groups of 6 New Zealand White rabbits (0.79-4.38 kg, sex not stated) received i.v. (right ear) either:

1) 0.9% Sodium Chloride Injection, USP;
2) ASL-8052 100 mg/ml formulation (lot No. 864-7) (Vehicle: 10% Propylene Glycol USP, 10% Alcohol USP and 0.1 M Acetate buffer in Water for Injection USP) diluted with 0.9% Sodium Chloride Injection USP to a final concentration of 10 mg/ml.

Venous Irritation: 0.9% Sodium Chloride was infused for 6 hours at a rate of 1.0 ml/min. ASL-8052, 10 mg/ml, was infused (syringe pump) for 6 hours through a teflon catheter inserted into the marginal ear vein of the right ear (catheter was removed immediately after infusion) at rates of 0.02, 0.05, 0.1, 0.2, 0.5 or 1.0 ml/min resulting in ASL-8052 delivery rates of 0.2, 0.5, 1.0, 2.0, 5.0 and 10 mg/min, respectively.

Rabbits were treated and observed for venous irritation at approximately 1 and 24 hours after treatment. After the 24 hour grading period rabbits were euthanized (CO_2) and each ear prepared for histologic evaluation.

Macroscopically, 2 to 10 mg/min caused dose-related erythema that ranged in severity from well defined to severe. The slight to well defined erythema seen at lower doses, although significantly more than seen with saline, was considered by the sponsor to be biologically insignificant. Only very slight edema was seen at 5 and 10 mg/min.

Microscopically, saline caused very slight irritation and ASL-8052 caused increasing irritation the severity of which was dose-related. The irritation caused by doses of 2, 5, and 20 mg/min ranged from slight to marked and was significantly more than seen with saline. Observations included perivenous hemorrhage, perivenous edema, perivenous inflammatory infiltrate, leucocyte pavementing, and occluding and non-occluding thrombi.

Summary: ASL-8052 100 mg/ml Vehicle (10% Propylene Glycol USP, 10% Alcohol USP and 0.1 M Acetate buffer in Water for Injection USP) was diluted with 0.9% Sodium Chloride Injection USP to a final concentration of 10 mg/ml and infused at rates of 0.2 to 10 mg/min into the marginal ear vein of rabbits of 6 hours. Infusion at rates of 2 mg/min or higher caused substantial venous irritation.

b. Dog:

(1). Undiluted ASL-8052 in 10% Propylene Glycol-10% Alcohol: Infused i.v. for 3 Days:

Twelve beagle dogs (Ridglan Farms, Inc., Mt. Horeb, WI) were divided into two groups of 6 (3/sex) dogs each. Either ASL-8052 (100 mg/ml in Propylene Glycol USP, 10%; Ethanol USP, 10%; Sterile Water for Injection USP, q.s. with 0.1 M acetate buffer, pH 5.0. Lot no. 736-34) of 0.9% Sodium Chloride Injection USP was infused (Cormed, mode ML6 pump) into a previously cannulated (silastic tubing) jugular vein at a rate of 0.3 ml/kg/hr (500 mcg/kg/min for ASL-8052) for 72 hours.

No systemic toxicity was observed. At necropsy all saline-treated dogs had patent jugular veins with small thrombi present in 2/6 dogs. In contrast, 5/6 ASL-8052 treated dogs had large dark brown thrombi surrounding the cannulae and filling most of the vein. Histologically, saline treated dogs had mild to marked, mechanically-induced damage to the vein walls and non-occluding mural thrombi. The veins of 5/6 ASL-8052 treated dogs had occluding thrombi, mild to severe phlebitis, and inflammation of surrounding tissues.

The Sponsor concludes "Undiluted ASL-8052 infused for three days into the jugular veins of Beagle dogs produced mild to severe local irritation as characterized by mild to severe thrombophlebitis with extension of inflammation into surrounding tissues.

(2). 1:5 and 1:10 Dilutions of ASL-8052 in 10% Propylene Glycol-10% Alcohol: Infused 24 or 48 Hours:

In study T8052-031, infusion of an undiluted formulation of ASL-8052 into the jugular vein of dogs for 72 hrs caused mild to severe thrombophlebitis, therefore, this study was performed to determine the local effects of 1:5 and 1:10 dilutions (using 5% Dextrose USP) of the same formulation of ASL-8052 (different lot No 767-49)used in the previous study. Beagle dogs were prepared for i.v. infusions as in study T8052-031. Separate groups of 4-6 dogs each were infused with:

1) 5% dextrose at 1.8 ml/kg/hr X 48 hrs; 6 dogs.
2) Vehicle, 0.3 ml/kg/hr X 24-48 hrs; 4 dogs. The intended duration was 24 hrs but due to technical reasons 2 dogs were infused for 48 hours.
3) ASL-8052, 20 mg/ml at 0.9 ml/kg/hr X 24 hr; 300 mcg/kg/min. 4 dogs.
4) ASL-8052, 10 mg/ml at 1.8 ml/kg/hr X 24 hr; 300 mcg/kg/min. 6 dogs.
5) ASL-8052, 10 mg/ml at 1.8 ml/kg/hr X 48-72 hr; 300 mcg/kg/min. 4 dogs. The intended duration was 48 hrs but due to technical reasons 2 dogs were infused for 72 hours.

ASL-8025 was, therefore, always infused at a rate of 300 mcg/kg/min. This was achieved by infusing the higher concentration at a slower volume rate. In the previous study the undiluted ASL-8052 formulation (100 mg/ml) was infused at a volume rate of 0.3 ml/kg/hr thus delivering ASL-8052 at 500 mcg/kg/min.

There were no sign of systemic toxicity throughout this study.

SUMMARY OF GROSS NECROPSY AND MICROSCOPIC IRRITATION SCORES

	HOURS OF INFUSION								
	24 Hr			48 Hr			72 hr		
	THROMBI	OCCLUDE	SCORE	THROMBI	OCCLUDE	SCORE	THROMBI	OCCLUDED	SCORE
5% Dextrose	-	-	-	4/6	0/6	3.2	-	-	-
Vehicle	2/2	0/2	2.0	2/2	2/2	5.0	-	-	-
ASL, 20 mg/ml	2/4	0/4	4.3	-	-	-	-	-	-
ASL, 10 mg/ml	2/6	1/6	3.0	1/2	0/2	4.0	2/2	2/2	5

THROMBI = Number of dogs with thrombi in jugular vein at necropsy.
OCCLUDE = Number of dogs in which jugular vein was totally occluded by thrombus.
SCORE = Irritation score. After histologic examination of each vein an irritation score from 1 to 6 was assigned. A score of 1 was comparable to an uncatheterized vein and a score of 6 indicated very severe thrombophlebitis and necrosis

SUMMARY MICROSCOPIC IRRITATION SCORES

	HOURS OF INFUSION													
	24 Hr					48 Hr				72 hr				
	1	2	3	4	5	2	3	4	5	2	3	4	5	
5% Dextrose						2/6	2/6	1/6	1/6					
Vehicle		2/2							2/2					
ASL, 20 mg/ml				3/4	1/4									
ASL, 10 mg/kg	2/6	1/6		1/6	2/6		1/2		1/2				2/2	

There were only 2 out of the 24 dogs in this study in which the infused vein was comparable to a non-cannulated vein. These two dogs were in the group infused with ASL-8052 at 10 mg/ml for 24 hours. All other dogs had some evidence of irritation, due either to the catheter or infused agent, ranging from a mild reaction to severe thrombophlebitis (none had necrosis or a score of 6). Although the Sponsor states that "After 24 hours there was probably no meaningful difference between the groups infused with vehicle, ASL-8052 1:10 of ASL--8052 1:5", it does appear that the 20 mg/ml solution (4/4 dogs had scores of 4 or more) was more irritating than the 10 mg/ml solution (3/6 dogs had scores of 4 or more). The experiments also indicate that the severity of the response is related to the duration of infusion. The undiluted vehicle was more irritating the 5% dextrose and may account for much of the irritation seen with ASL-8052. This experiment is not complete enough to clearly conclude the degree of contribution of vehicle and ASL-8052 to the induction of severe thrombophlebitis. It must, therefore, be concluded that i.v. infusion of the ASL-8052 formulation even when diluted 1:10 with 5% dextrose to a concentration of 10 mg/ml into dog jugular veins for 24 to 72 hours causes slight to severe thrombophlebitis with occasional occlusion of the infused vein. The incidence and severity of these findings increased with increasing infusion durations. The Sponsor states "Lung thrombi occurred in one or two dogs from each group. There was no relationship between occurrence of lung thrombi and material infused or duration of infusion. All thrombi observed occurred in vessels less than 0.5 mm diameter and there was no evidence of infarction associated with thrombi". It is not clear whether these pulmonary thrombi represent emboli from the cannulated vein or arose during sacrifice (anesthesia and exsanguination), but absence of infarction suggests the latter.

(3). ASL-8052 vs. Methyl Ethyl Ketone - A Contaminant

In previous studies ASL-8052 infused for 72 hours caused mild to sever thrombophlebitis resulting in occluded veins. The incidence of these findings was reduced when an ASL-8052 formulation was diluted 1:10 (5% Dextrose USP) and infused for 24-72 hours. Furthermore, the vehicle may be in part responsible for the local effects. The present study attempts to determine if ASL-8052 itself or a contaminant, Methy Ethyl Ketone (MEK), causes local thrombophlebitis when infused for 72 hours.

One day prior to initiating drug treatment each dog was surgically prepared by inserting a catheter (silastic) into a jugular vein. The catheter was attached to a pump (Cormed, model ML6) held in a jacket and each dog was infused with 0.9% Sodium Chloride, USP at a rate of 0.3 ml/kg/hr. There were 4 treatment groups:

1) ASL-8052 (MEK-free) in 0.9% Sodium Chloride USP at 100 mg/ml (pH adjusted to 5.0) (lot No. 815-5D;
2) ASL-8052 (100 mg/ml in Propylene Glycol USP, 10%; Ethanol USP, 10%; Sterile Water for Injection USP, q.s. with 0.1 M acetate buffer, pH 5.0. Lot no. 815-5A). This formulation contains MEK as a contaminant at 111 ppm;
3) Methyl Ethyl Ketone (MEK) dissolved in 0.9% Sodium Chloride, USP at a concentration of 1.64 mg/ml (lot No. 815-5E); and
4) 0.9% Sodium Chloride, USP (lot No. B2K097C).

	Normal Saline	MEK[a]	ASL-8052 in Saline	ASL-8052 Formulation
No. Dogs (Mongrel)[b]	6	4	6	6
Concentration, mg/ml	-	1.64	100	100
Infusion Rate, ml/kg/hr	0.3	0.3	0.3	0.3
Dose, mcg/kg/min	-	8.2	500	500
Infusion Duration, hours	72	72	72	72
Jugular Vein Patent	6	4	2	0
Jugular Vein Occluded	0	0	4	6
Non-Occluding Red Thrombi	1	0	0	0
Non-Occluding Dark Brown Thrombi	0	1	0	0
Occluding Dark Brown Thrombi	0	0	4	6
Subintimal Hemorrhage, vein wall	1	0	1	0
Edema	0	0	1	5
Non-inflammatory reactions	3	2	0	0
Thrombophlebitis	3	2	6[c]	6[d]
Microscopic Irritation Score, mean	3.3	3.3	4.5[c]	6.0[d]
Standard deviation	1.0	0.5	1.2	0.0
Lesions on Lungs	0	1	1	0

[a] Concentration and dose of MEK indicated in this table were taken from Sponsors text. Sponsor's Table 1 indicates concentration and dose of MEK at 1.5 mg/ml and 7.5 mcg/kg/min, respectively.

[b] Sponsor states that 13 male and 13 female mongrel dogs were selected for this experiment. There is no indication as to the number of dogs/sex in each group, why only 22 of the 26 selected dogs were used or why the groups are not equal in size (only 4 dogs in the MEK group).

[c] Frequent extension of inflammation into surrounding connective tissue and occasional signs of vein wall necrosis.

[d] Frequent extension of inflammation into surrounding connective tissue. Necrosis of vein wall and surrounding tissue observed frequently.

Summary: In dogs, infusion of normal saline or methy ethyl ketone in saline into a cannulated jugular vein for 72 hours produced a non-inflammatory reaction to mild thrombophlebitis, probably due to mechanical irritation of the catheter. Infusion with ASL-8052, either in saline or in its final formulation, caused severe irritation characterized by extensive thrombophlebitis and necrosis of the vein wall with frequent extension of the inflammation, and occasionally necrosis, to the surrounding tissue. Although the venous irritation may have been more severe in the ASL-8052 Formulation group than in the ASL-8052 Saline group, it is clear that the major cause of the irritation is ASL-8052 and not the vehicle or the methy ethyl ketone contaminant. There was no evidence of ASL-8052-induced pulmonary lesion which might result from increased numbers of emboli.

5. Reproduction Studies: Segment II - Teratology:

a. Rat:

Dose Range-Finding Study:

Separate groups of 5 pregnant Charles River COBS CD rats received intravenously (lateral tail vein) either vehicle(10% Propylene Glycol USP, 10% Alcohol USP, Sterile Water For Injection USP and 0.1 M Acetate buffer), or Brevibloc at 1.0, 5.0, 10, 12.5, or 15 mg/kg/min for 30 min once a day on days 6 through 15 of gestation. Total daily doses of Brevibloc were, therefore, 30, 150, 300, 375 or 450 mg/kg. The Brevibloc formulation was appropriately diluted with 0.9% NaCl. Sponsor indicates that Brevibloc was initially (first 8 days) diluted to 5, 25, 50, 62.5 and 75 mg/ml and administered at 0.2 ml/kg/min (6 ml/kg/day) but later (last 5 days) diluted to 1.0, 5.0, 10.0, 12.5 and 15.0 mg/ml and administered at 1 ml/kg/min (30 ml/kg/day).(each at a rate of 0.2 ml/kg/min, i.e. 6 ml/kg/day).

NOTE: Since treatment was from day 6 to 15, i.e. 10 days, it is unclear how long each dilution was actually administered. It is also unclear whether 0.9% NaCl, vehicle or diluted vehicle was used in the control animals.

Teratology Study:

Species/Strain: Female Charles River COBS CD rats (12 wks old, 218-287 gm at mating).
Study Dates: 08/08/83 to 09/07/83
Test Substance/Formulation: ACC supplied ampules containing 10 ml of a ASL-8052 formulation: 100 mg/ml in 10% Propylene Glycol USP, 10% Alcohol USP, Sterile water for Injection USP and 0.1 M Acetate buffer (pH 5). Appropriate dilutions were made by IRDC using 0.9% NaCl for Injection USP to achieve concentrations of 1.0, 3.0 and 10 mg/ml. ACC requested that on the first day of dosing sufficient dosing solutions be prepared to last the entire study. This was not done until day 2 of dosing. Analysis by ACC (samples shipped frozen) indicated the the amount of ASL-8052 in the dosing solutions ranged from 96.1% to 106.0% of theoretical and none was detected in either control solution. These results indicate that the solutions were prepared properly but do not indicate whether the compound was stable in solution over the dosing interval.

Route and Schedule of Administration: Infused (syringe pump) i.v. (lateral tail vein) at a constant rate of 1 ml/kg/min X 30 minutes once a day on Days 6 through 15 of pregnancy.
Doses: 0.0 (control), 1.0, 3.0 and 10.0 mg/kg/min (0.0, 30, 90 and 300 mg/kg/day). There were 2 control groups; a 0.9% NaCl for injection USP and a vehicle control (vehicle diluted 1;10 with 0.9% NaCl).
Number of Animals: 25 mated females/group.
Cesarean Section: Day 20 of pregnancy.
Plasma Levels: Blood samples (0.5 ml, orbital sinus) obtained immediately upon infusion termination from 6 rats/group on Days 6 & 8, 1 rat/group on Day 11, 5 rats/group on Day 12, and 7 rats/group on Day 15, were processed, frozen and shipped to ACC for ASL-8052 and ASL-8123 analysis.

RESULTS: Maternal Observations: Mortalities: 0/25 in the vehicle control, 1/25 in the 0.9% NaCl control, 2/25 in the 1 mg/kg/min group, 1/25 in the 3 mg/kg/min group and 10/25 in the 10 mg/kg/min group. Deaths. The female in the NaCl control group died on day 6 prior to infusion. In the remaining group the majority of deaths occurred during infusion and about half died on the first day of dosing (Day6). The others died on Days 7 - 14 of Pregnancy. Cause of deaths was not determined. Behaviour: Unremarkable in the 1 & 3 mg/kg/min groups. Observations in the 10 mg/kg/min group included convulsions (and gaping in 2 cases) in 3/10 deaths and in 1 survivor, and reduced activity following infusion in 4 rats. Body Weight Gains: there were no significant and dose related drug effect on body weight gains either before, during or after dosing. Clinical Findings: No drug-related effects. Necropsy: No drug related findings. Normally developing embryos were present in the gravid animal that died on study. Spontaneous Deliveries: None. No Gravid at Cesarean Section: 21/24 in NaCl control, 25/25 in vehicle control, 21/23 in 1 mg/kg/min, 19/24 in 3 mg/kg/min, and 15/15 in 10 mg/kg/min. There were no significant drug effects on viable fetuses/dam, postimplantation loss/dam, total implantations/dam, corpora lutea/dam, % pre-or post-implantations loss, mean fetal body weight or sex distribution.
Fetal Morphology:

	DOSAGE GROUP				
	NaCl	Vehicle	1.0	3.0	10
No. of Litters Examined	21	25	20	19	15
No. of Fetuses Examined Externally	263	337	267	236	179
No. of Fetuses Examined Viscerally	90	117	93	81	63
No. of Fetuses Examined Skeletally	173	220	174	155	116

There were no statistically significant or dose-related differences in the incidence of external, visceral or skeletal malformations, or developmental variations between drug treated and control animals.

	CONCENTRATION OF ASL-8052 IN WHOLE BLOOD, mcg/ml			
	DAY 6	DAY 8	DAY 11-12	DAY 15
Saline	0.00 ±0.00 (4)	0.00 ±0.00 (5)	0.00 ±0.00 (6)	0.00 ±0.00 (5)
1 mg/kg/min	0.18 ±0.16 (3)	0.83 ±1.23 (6)	2.32 ±0.37 (6)	1.13 ±1.12 (6)
3 mg/kg/min	2.55 ±2.83 (4)	2.27 ±3.13 (6)	4.72 ±4.64 (6)	1.74 ±3.41 (6)
10 mg/kg/min	11.8 ±8.66 (2)	7.56 ±13.1 (4)	1.82 ±0.97 (5)	4.20 ±7.83 (5)

Mean ±S.D.

	CONCENTRATION OF ASL-8123 IN WHOLE BLOOD, mcg/ml			
	DAY 6	DAY 8	DAY 11-12	DAY 15
Saline	00.0 ±00.0 (5)	00.0 ±00.0 (5)	00.0 ±00.0 (5)	00.0 ±00.0 (7)
1 mg/kg/min	28.5 ±11.7 (6)	24.8 ±10.0 (6)	18.7 ±09.3 (5)	30.5 ±10.6 (6)
3 mg/kg/min	65.0 ±33.7 (6)	85.7 ±36.4 (6)	85.4 ±33.4 (6)	119.0 ±28.9 (7)
10 mg/kg/min	232. ±66.1 (4)	350. ±95.6 (4)	308. ±119 (5)	354. ±99.9 (5)

Mean ± S.D.

From the Sponsors Summary: "Blood concentrations of ASL-8052 and its metabolite ASL-8123 were dose dependent and exhibited no accumulation during the study period. Average concentrations for ASL-8052 were 1.25, 2.85, and 5.24 mcg/ml for the ASL-8052 1, 3, and 10 mg/kg/min groups, respectively. Average concentrations of ASL-8123 were 25.6, 88.8 and 313 mcg/ml, respectively. Further analysis suggested the presence of two subgroups of animals based on relative concentrations of ASL-8052 and its metabolite. These subgroups were apparently rhe result of different rates of ASL-8052 esterase activity. The "slow" group, consisting of 36% of the animals analyzed, exhibited ASL-8052 levels which were 13 to 22 times higher than those in the "fast" group. Conversely, the blood concentrations of ASL-8123 in the "slow" group were approximately 60% to 70% of those in the "fast" group".

Summary: In the rat intravenous infusion of ASL-8052 at up to 3.0 mg/kg/min for 30 min a day on days 6 through 15 of gestation was not maternotoxic, fetotoxic, or teratogenic. Infusion of 10 mg/kg/min caused excessive maternal mortalities (probably due to excessive pharmacodynamic effects) but was not fetotoxic or teratogenic.

b. Rabbit:

Dose Range-Finding Study:

Separate groups of 5 pregnant New Zealand White Rabbits received intravenously (marginal ear vein) either vehicle(0.9% NaCl), or Brevibloc at 1.0, 2.5, 5.0, 7.5, or 10 mg/kg/min for 30 min (each at a rate of 1 ml/kg/min) once a day on days 6 through 18 of gestation. Total daily doses of Brevibloc were, therefore, 30, 75, 150, 225 or 300 mg/kg.

Mortalities: None in the control or 1 mg/kg/min groups. 2/5 in the 5 mg/kg/min dose group and all rabbits (5/5) in the 7.5 and 10 mg/kg/min dose groups. All died on the first day of dosing and apparently before the infusion was completed. The cause of death could not be determined. The only reported clinical sign in some (incidence dose-related) of these animals was "wet, clear or red matting of the haircoat around the nose and/or mouth." One of the 5 animals in the 2.5 mg/kg/min group was sacrificed in extremis on day 16. Clinical signs included " inability to use hindlimbs, hair loss from ventral neck and tail, wet, matted haircoat in the anogenital area, ventral abdomen, dorsal posterior and hindlimbs, and swelling in the anogenital area. Necropsy revealed yellow fluid in the uterus and cervix.

Two of the remaining 4 rabbits in the 2.5 mg/kg/min group aborted, one on day 27 and one on day 29 of gestation. Body weight ganins were reduced in the 1.0 an 2.5 mg/kg/min dose groups during the treatment period (days 6-18)

The number of gravid does examined at necropsy in the control, 1.0, 2.5 and 5.0 mg/kg/min dosage groups were 4, 5, 2 and 3, respectively. There were no differences between groups in terms of number of corpora lutea/doe and there was no difference between the control group and the 2.5 mg/kg/min dosage group in therms of viable fetuses/doe, postimplantations loss/doe, total implantations/doe, or % preimplantation and postimplantation loss (group means). In the 1.0 and 5.0 mg/kg/min dosage groups there was a decrease in the number of viable fetuses/doe due largely to increases in preimplantations losses and partly to increases in postimplantation losses (esp. in the 1.0 mg/kg/min group).

"Based on these results, dosage levels of 0.5, 1.0 and 2.5 mg/kg/min were selected for a definitive teratology study in rabbits with ASL-8052."

Teratology Study:

Species/Strain: Female New Zealand White Rabbits (6 to 7 mo. old at insemination)

Study Dates: 09/07/83 to 10/07/83
Test Substance/Formulation: ACC supplied ampules containing 10 ml of a ASL-8052 formulation: 100 mg/ml in 10% Propylene Glycol USP, 10% Alcohol USP, Sterile water for Injection USP and 0.1 M Acetate buffer (pH 5). Appropriate dilutions were made by IRDC using 0.9% NaCl for Injection USP to achieve concentrations of 0.5, 1.0 and 2.5 mg/ml. Dilutions were prepared fresh daily. Analysis by ACC (samples shipped frozen) indicated the the amount of ASL-8052 in the dosing solutions ranged from 100.2% to 105.2% of theoretical and none was detected in either control solution (vehicle or 0.9% NaCl).
Route and Schedule of Administration: Infused (syringe pump) i.v. (marginal ear vein) at a constant rate of 1 ml/kg/min X 30 minutes once a day on Days 6 through 18 of pregnancy.
Doses: 0.0 (control), 0.5, 1.0 and 2.5 mg/kg/min (0.0, 15, 30 and 75 mg/kg/day). There were 2 control groups; a 0.9% NaCl for injection USP and a vehicle control (vehicle diluted 1;10 with 0.9% NaCl).
Number of Animals: 15 mated females/group.
Cesarean Section: Day 29 of pregnancy.
Plasma Levels: Blood samples (0.5 ml, marginal vein on opposite ear) obtained immediately prior to infusion termination from 8 does/group on Days 6 & 13, and from the remaining 7 does/group on Day 9, 18, were processed, frozen and shipped to ACC for ASL-8052 and ASL-8123 analysis.

RESULTS:

ASL-8052 (mcg/ml) in RABBIT WHOLE BLOOD

Dose mg/kg/min	Day 6	Day 9	Day 13	Day 18	Mean
Saline	0.0	0.0	0.0	0.0	0.0
0.5	2.1	1.1	1.6	3.0	1.9
1.0	4.2	2.4	2.8	6.0	3.8
2.5	8.4	4.9	9.8	18.4	10.0

ASL-8123 (mcg/ml) in RABBIT WHOLE BLOOD

Dose mg/kg/min	Day 6	Day 9	Day 13	Day 18	Mean
Saline	0.0	0.0	0.0	0.0	0.0
0.5	22.7	20.9	24.5	20.1	22.1
1.0	39.8	42.0	43.5	40.3	41.4
2.5	95.3	99.6	97.4	95.7	97.0

There was a dose-dependent increase in peak blood levels of both ASL-8052 and its major acid metabolite, ASL-8123 as the dose of ASL-8052 was increased from 0.5 to 2.5 mg/kg/min.

Plasma levels of ASL-8052 but not ASL-8123 tended to be higher on day 18 than on day 1, 9 or 13. Sponsor indicates that this was not statistically significant.

Plasma levels of ASL-8123 were 10 to 20 times higher than ASL-8052 except on day 18 when levels of ASL-8123 were 5 to 7 times higher than ASL-8052. The ratio of the two compounds was not dose-related within the range of doses studied.

Maternal Observations: Mortalities:

			mg/kg/min/day		
	Saline	Vehicle	0.5	1.0	2.5
Number on Study	15	15	15	15	15
Died	1	2	2	2	3
(day)	(17)	(10, 11)	(24, 27)	(24, 29)	(16, 21, 22)
Sacrificed (day)	0	0	0	1(19)	0
Aborted prior to death	0	0	1	0	2
Aborted prior to C. section (day)	1 (22)	0	1 (28)	0	0

Cause of deaths could not be determined. The most common clinical signs in animals that died or were sacrificed included (number of animals affected): stained/wet/matted hair in the anogenital area (6), nasal discharge (5), incapacity of hind-limbs (4), abortion (3), loss of bladder control (3, all with incapacity of hind-limbs), hair loss (particularly in the dorsal anterior region)(2), ocular discharge (2), and emaciations (2). These signs were seen in controls (saline and vehicle) as well as drug-treated animals. Observations in single animals included dark red urine (vehicle), respiratory rales (0.5 mg/kg/min), soft stool (1.0 mg/kg/min), inability to breathe through nose (2.5 mg/kg/min) and unsteady movement (2.5 mg/kg/min). The most common gross necropsy firdings in control and drug-treated animals included (number of rabbits): congested lungs (5) fluid in thoracic

cavity (5), consolidated lungs, (4), fluid in abdominal cavity (2), and abdominal adhesions (2); findings in drug-treated animals only included: pulmonary/thoracic adhesions (3), mucoid material in small intestine (3), fibrous material covering lungs or diaphragm (3), gelatanous material in large intestine (2), and fibrous/mucoid material covering urinary bladder (2). It is unlikely that these findings in drug-treated rabbits were due to drug since similar findings were seen in control animals and drug-treated animals that survived to scheduled sacrifice (see table below). Only 1 rabbit died during treatment period and this occurred during infusion of 2.5 mg/kg/min and may have been drug related. There were no clinical signs of toxicity in this animal and necropsy findings included congested lungs, fluid in thoracic cavity and mucoid yellow material in small intestine.

NUMBER OF RABBITS SURVIVING TO CESAREAN SECTION (Day 29) WITH CLINICAL SIGNS AND NECROPSY OBSERVATIONS

	SALINE	VEHICLE	0.5	1.0	2.5
No. Survivors	14	13	13	12	12
No. Without Clinical Signs	7	2	6	6	3
No. Without Gross Lesions	9	7	7	9	9
Hair Loss/Scabbing	4	7	4	3	4
Nasal Discharge, clear/white/yellow	4	5	3	4	6
Ocular discharge	2	4	1	0	1
Anogenital Area, stained/wet/matted	1	1	0	1	2
Soft Stool	0	3	0	0	0
Incapacity of Hind Limb(s)	0	0	0	0	2
Aborted	1	0	1	0	0
Lungs, dark pin-point foci	1	0	1	0	1
Lungs, Congested	1	4	3	1	2
Lungs, Consolidated	1	0	1	0	1
Thoracic Cavity, Fluid, clear	0	3	3	1	0
Abdominal Cavity, Fluid, clear	0	2	0	0	1
Oviduct Hydroceles	0	1	0	1	0
Kidney, Pitted surface	0	0	0	1	1
Abscesses, neck/axilla	0	0	0	0	1
Abdominal Adhesions	1	0	0	0	0

Thus, none of the clinical and necropsy findings in animals that died or survived appear drug related. They appear to be related to the trauma of the treatment procedure and are compatible with spinal cord injury and systemic infection.

BODY WEIGHT CHANGES (grams) OF DOGS

TREATMENT DAYS	SALINE	VEHICLE	0.5	1.0	2.5
0-6	+85	+87	+110	+56	+74
6-18	-12	+58	+101	-6	-48
18-29	+151	+181	+96	+189	+147
0-29	+260	+326	+325	+372	+199

During the treatment period (days 6-18) does in the 2.5 mg/kg/min group lost weight resulting in a significant lower weight gain during gestation (day 0-19) than in the other groups.

OBSERVATIONS AT CESAREAN SECTION

OBSERVATION	SALINE	VEHICLE	0.5	1.0	2.5
Gravid Does Examined	13	13	11	12	12
Does with Viable Fetuses	13	13	10	11	9
Does with Resorptions Only	0	0	1	1	3
Total Viable Fetuses	83	93	90	71	59
Total Non-Viable Fetuses	0	1	0	1	0
Viable fetuses/Doe	6.4	7.2	8.2	5.8	4.9
(Viable fetuses/Viable litter)	(6.4)	(7.2)	(9.0)	(6.5)	(6.6)
Corpora Lutea/Doe	12.9	11.7	10.9	13.5	11.3
Total Implantations/Doe	7.2	8.2	9.2	7.1	7.1
Post Implantations Loss/Doe	0.8	1.1	1.0	1.3	2.2
Preimplantation Loss, %	44.0	29.6	17.3	43.6	38.1
Postimplantation Loss, %	11.7	13.1	10.9	17.6	30.6
Mean Fetal Weight, gm	40.1	39.1	35.0	41.8	39.5
Early Resorptions, Total	10	6	10	12	26
No. Litters	6	5	2	5	7
Mean/doe	0.8	0.5	0.9	1.0	2.2
Late Resorptions, Total	1	7	1	2	0
No. Litters	1	4	1	2	0
Mean/Doe	0.1	0.5	0.1	0.2	0

ASL-8052 at 2.5 mg/kg/min significantly increased post-implantation losses primarily by increasing early resorptions. The compound had no effect on late resorptions, the incidence of non-viable fetuses, or average fetal weight of viable fetuses.

MALFORMATIONS

OBSERVATION	SALINE	VEHICLE	0.5	1.0	2.5
No. Fetuses Examined, Externally, Viscerally and Skeletally	83	94	90	78	59
No. With Malformations	6	7	2	7	3
(No. Litters)	(4)	(5)	(2)	(4)	(3)
No. with 1 Malformation	5	6	2	5	0
No. with 2 Malformations	1	0	0	2	2
No. with 11 Malformations	0	1	0	0	1

There was no drug induced increase in the incidence or number of malformations or an increase in developmental variations. Therefore, in the rabbit ASL-8052 was not teratogenic at i.v. doses up to 2.5 mg/kg/min X 30 min/day (75 mg/kg/day) administered on days 6 through 18 of pregnancy.

6. In Vitro Interaction with Human Blood:

Incubation of human red blood cells or human plasma with various saline dilutions of the ASL-8052 formulation caused hemolysis and protein flocculation at concentrations of 10 mg/ml and above but not at 5 mg/ml or lower.

a. ASL-8052 10% Propylene Glycol-10% Alcohol:

The ASL-5082 formulation (100 mg/ml) contained sodium acetate, acetic acid, 10% Propylene Glycol USP, and 10% Alcohol USP. NaOH and HCl were used to titrate to a pH of 5. Dilutions (concentrations) tested: 1:100 (1 mg/ml), 1:50 (2 mg/ml), 1:20 (5 mg/ml), 1:10 (10 mg/ml), 1:5 (20 mg/ml), 1:2 (50 mg/ml), and undiluted (100 mg/ml).

Human blood from 4 donors was collected. The plasma was separated for the plasma protein flocculation test and the packed RBCs were washed with saline and used for the hemolysis test (osmotic fragility). All samples had less tha 5% hemolysis with 0.5% NaCl and more than 90% hemolysis with 0.3% NaCl.

There was no significant plasma protein flocculation at any of the ASL-8052 concentrations.

There was no significant hemolysis at ASL-8052 concentrations up to 5 mg/ml. Higher concentrations caused hemolysis the degree of which was concentration-dependent. The maximal hemolysis at an ASL-8052 concentration of 100 mg/ml was about 70%.

These results are similar to those obtained with an ASL-8052 formulation in 70% propylene glycol, 30% alcohol and water (see T8052-017).

b. ASL-8052 in 25% Glycerine-60% Alcohol:

After passing an osmotic fragility test, blood (collected from 4 human donors) was separated into plasma and packed cells. The plasma was used for the plasma protein flocculation test and the packed were washed with saline and used in the hemolysis test. Washed cells and plasma were mixed in separate tubes with non-diluted 25% ASL-8052 in 20% Glycerine USP and 60% Alcohol USP (lot No. 899-26) or 0.9% Sodium Chloride dilutions yielding concentration of 1 to 100 mg/ml of ASL-8052

% HEMOLYSIS

Distillied Water	0.9% Sodium Chloride	ASL-8052, mg/ml 1.0	2.0	5.0	10	20	50	100	250
100	0	0.4	0.9	5.5	19.6	55.9	60.5	77.4	51.9

% PLASMA PROTEIN FLOCCULATION

10% TCA*	0.9% Sodium Chloride	ASL-8052, mg/ml 1.0	2.0	5.0	10	20	50	100	250
100	0	0.3	0.1	0.2	0.7	1.0	1.5	8.1	8.5

* Trichloroacetic Acid

Summary: A 1:50 dilution (5 mg/ml) of the 25% ASL-8052 Formulation was the highest concentration that did not produce substantial hemolysis or plasma protein flocculation in human blood. Concentrations of 20 mg/ml to 250 mg/ml (non-diluted formulation) produced 52% to 77% hemolysis while concentrations of 100 and 250 (non-diluted) mg/ml produce 8% to 9% protein flocculation. The degree of protein flocculation was substantially less than the 100% seen with the positive control (TEA). The hemolysis and plasma protein flocculation produced by this formulation were similar to those produced by equal concentrations of 10% ASL-8052 formulation in previous studies (see T8052-017 & T8052-027).

c. ASL-8052 in 0.9% NaCl - Acetate Buffer:

Earlier in vivo studies indicated that ASL-8052, when infused intravenously at high concentrations, caused phlebitis and thrombosis at the infusion site. The purpose of this study was to determine whether ASL-8052 has direct effects on blood coagulation or platelet function when tested in vitro. The study was conducted in a blinded manner with the investigator not knowing which of the two solutions supplied by American Critical Care contained Vehicle Placebo (0.25 M acetate buffer in 0.9% Sodium Chloride Injection USP, pH adjusted to 5 with NaOH) or ASL-8052 (25%, i.e., 25 mg/ml in above vehicle).

Blood was collected from 6 subjects and both Vehicle and 25% ASL-8052 were tested using blood from each subject. One volume of either Vehicle or 25% ASL-8052 was diluted with 4 volumes of either whole blood, plasma or platelet rich plasma, depending on the assay being performed. The following assays were performed: Partial thromboplastin time, prothrombin time, thrombin time, fibrinogen, clot retraction, platelet aggergation, and platelet adhesiveness.

Partial thromboplastin, prothrombin and thrombin times were all greatly prolonged by ASL-8052 and slightly prolonged by Vehicle. The vehicle effect is possibly a dilutional effect.

Fibrinogen concentrations were normal to slightly decreased with Vehicle and normal to moderately decreased with ASL-8052.

Clot retraction was unaffected by Vehicle and could not me measured with ASL-8052 due to severe hemolysis.

Platelet adhesiveness was moderately to severely decreased with bothe Vehicle and ASL-8052.

7. Ocular Toxicity - Rabbit:

a. 1%, 3% and 10% Esmolol in Water:

Draize Test: Four(4) separate groups of 4 male rabbits each received into the conjunctival sac of the right eye a single instillation of 0.1 ml of either sterile water or esmolol in sterile water at 1%, 3% or 10%. Sponsor states that esmolol in stable in water over the period of instillation. The pH of the solutions varied from 5.7 (sterile water) to 5.3 (10% esmolol). Left eye served as untreated control. Eyes were examined immediately (within 1 hr) and on days 1, 2, 3, 4 and 7 post treatment. Sodium fluorescein and UV light were used on days 3 and 7 to visualize damage. Treatment had no effect of body weights nor was there any evidence of irritation at 1 hr or on any post-treatment days. Slight transient ocular reactions (immediate onset with reversal within 10 min) consisting of lid closure, blinking, and mucoid discharge were seen with the 3% and 10% solutions (incidence was concentration-dependent and all 4 rabbits showed effect at 10%).

b. 0.5%, 1%, and 3% Esmolol in Acetate Buffer with Benzalkonium (Ophthalmic Formulation) vs. Timoptic - 21 Days:

Six(6) separate groups of rabbits (6/sex/group) received into the conjunctival sac 3 times daily (at intervals of 3 hrs) for 21 consecutive days the instillation of 0.1 ml of either sterile 0.9% NaCl, ASL-8052 Ophthalmic Placebo (Acetate buffer 0.1 M, benzalkonium chloride 0.01%), 0.5% Timoptic, or ASL-8052 at 0.5%, 1.0% or 3.0% (in Ophthalmic Placebo). The contralateral untreated eye served as the control. There were no drug effects on behavior, body weight or other clinical signs. ASL-8052 was not irritating to the eye (cornea, iris conjunctivae) nor did it cause gross or histologic pathology.

c. 1%, 3% and 10 % Esmolol in Ophthalmic Formulation vs. Timoptic:

Six(6) separate groups of 6 male rabbits each received into the conjunctival sac of the right eye once every 3 hrs for a total of 3 applications 0.1 ml of either 0.9% NaCl Injection, ASL-vehicle (0.1 M acetate buffer, pH 5.0 with 0.01 benzalkonium chloride), 1% ASL-8502, 3% ASL-8502, 10% ASL-8502 or 0.5% Timoptic. The pH of the ASL-vehicle and the ASL-8502 solutions ranged from 4.7 to 5. The pH of saline and Timoptic was 6.5 and 7.0, respectively. The untreated left eye served as the control. Eye irritation was judged by use of a modified Draize technique, slit lamp, and indirect ophthalmoscopic exams.

Immediate Reactions seen on administration of the 3% and 10% formulations of ASL-8052 and 0.5% Timoptic included blinking and eye lid closure lasting 5 min or longer following each application. The incidence of these effects were concentration-dependent, and were of short durations lasting less than 10-15 minutes. No immediate effects were seen with saline, ASL-vehicle or 1% ASL-8052.
Draize and Slit Lamp Observations indicated slight and moderate irritation 1 hr after each application of 3% and 10% ASL-8052, respectively; the severity of the irritation was increased after the 3rd application. The irritation was characterized by conjunctival erythema, chemosis, and discharge. Fluorescein stain retention was seen 1 & 4 hr after the 3rd application of 10% ASL-8052 in 2/6 rabbits. Recovery was gradual and complete in 2 days.
Indirect Ophthalmoscopic exams were unremarkable 7 days after treatment.
Body weights were unaffected.

SUMMARY: These results indicate that ASL-8052 at 1% in saline or an acetate buffer (plus 0.01% benzalkonium chloride) did not produce immediate ocular irritation in the rabbit. In contrast, ASL-8052 at 3% and 10% either in saline or the acetate buffer caused slight and moderate immediate irritation, respectively, characterized by conjunctival erythema, chemosis, and discharge. These effects lasted less than 10 minutes. Sub-chronic (t.i.d. for 21 days) ocular administration of ASL-8952 at 0.5%, 1.0% or 3.0% was not irritating nor did it cause gross of histologic ocular pathology.

The ocular irritation produced by the 3% and 10% acetate buffer formulations of ASL-8052 was qualitatively similar, but slightly greater than that produced by the same concentration fo ASL-8052 dissolved in saline. The acid pH of the acetate buffer formulations can not account for the enhanced irritation since the pH of both the vehicle alone or the 1% formulation were similar (about 4.7). The enhanced irritation is not likely due to solution osmolarity since the osmolarity of the 3% acetate formulation was similar to that of saline. The 10% acetate formulation was, however, hyperosmolar.The Sponsor believes that the irritation produced by the 3% and 10% acetate formulation was a result of potentiation between vehicle and drug.

V. SUMMARY AND CONCLUSIONS:

PHARMACODYNAMICS:

beta-Adrenergic Receptor Blocking Activity:

In isolated spontaneously beating right atria, paced left atria and tracheal strips esmolol caused a concentration-dependent shift to the right of the isoproterenol concentration-effect curves with pA_2 values of 7.0 (32.4 mcg/ml), 6.5 (95.6 mcg/ml) and 5.2 (2293 mcg/ml), respectively. In isolated rabbit cardiac tissue and isolated canine purkinje fibers esmolol caused beta-receptor antagonism at concentrations of 10^{-7} M (32.4 mcg/ml) and above.

In anesthetized rabbits, esmolol infused i.v. at 1 mg/kg/min inhibited the positive chronotropic and the hypotensive actions of i.v. isoproterenol.

In anesthetized dogs, the infusion of esmolol at 50 mcg/kg/min resulted in steady state beta-receptor antagonism in 10-15 min. After infusion of esmolol for 3 hours recovery was rapid with 80% recovery in 12 min and essentially complete recovery in 20 min. At 100 mcg/kg/min, esmolol caused a dose-related, parallel shift to the right of the isoproterenol dose-response curve for both heart rate and contractile force with in vivo pA_2 values of 7.3 (17.8 mcg/kg/min) and 7.5 (20.4 mcg/kg/min), respectively. Ihfusions of esmolol at 5 to 160 mcg/kg/min caused dose-related inhibition of the heart rate (ID_{50} = 50 mcg/kg/min) and blood pressure (50% inhibition could not be obtained) effects of a standard dose of isoproterenol. Within the same dose range esmolol also produced dose-dependent inhibition of the effects of isoproterenol on left ventricular dp/dt and contractile force with ID_{50} values of 20 and 23 mcg/kg/min, respectively. Esmolol at 310 mcg/kg/min markedly reduced or abolished the effects of isoproterenol on blood pressure, left ventricular dp/dt, heart rate, coronary blood flow, myocardial oxygen consumption, and coronary vascular resistance. Responses to isoproterenol returned in 30 min. Esmolol was equally effective in inhibiting heart rate increases caused by sympathetic nerve stimulation (ID_{50} = 37 mcg/kg/min) and isoproterenol (ID_{50} = 50 mcg/kg/min).

The beta-adrenergic receptor blocking activity of esmolol in anesthetized dogs was not affected by ganglionic blockade.

Cardioselectivity:

In isolated guinea-pig tissues esmolol was cardio-selective since it was 24-71 times more potent in antagonizing atrial than tracheal beta-receptors. Propranolol was not cardio-selective and was about 4500 fold more potent than esmolol in blocking tracheal beta-receptors. In both the rabbit and dog, the positive chronotropic effects of isoproterenol were inhibited to a greater extent that its hypotensive action. At doses up to 160 mcg/kg/min in the dogs 50% inhibition of the hypotensive effect of isoproterenol could not be obtained. Based on ID_{50} values for inhibition of heart rate and perfusion pressure effects of isoproterenol in the anesthetize dog, esmolol and metoprolol were about 100 time more selective for cardiac than vascular beta-receptors and propranolol was non-selective.

In the anesthetized dog, esmolol was about 2 fold more potent in antagonizing inotropic than chronotropic responses to isoproterenol but this difference has essentially no clinical significance.

beta-Receptor Blocking Activity of Esmolol vs. Propranolol and Metoprolol:

In isolated atrial tissues, esmolol was approximately 40-140 times less potent than propranolol and 2-6 times less potent than metoprolol. Due to the compound's short duration of action (requiring constant i.v. infusion) and the much longer durations of action of propranolol and metoprolol (thus not suitable for continuous infusion studies) similar potency comparisons could not be made in intact animals.

Hemodynamic Effects of Esmolol.

In anesthetized rats, the minimum i.v. dose (administered over 45-60 sec) required to reduce heart rate was 1.5 mg/kg. Larger doses were required to decrease mean blood pressure (2.3 mg/kg), to reduce pulse pressure (2.6 mg/kg), to lower the ECG amplitude (5 mg/kg), and to slow respiratory rate (24.7 mg/kg). The magnitude of these effects were dose-related and tachyphalaxis did not occur. All effects lasted less than 10 minutes at doses up to 10 mg/kg. Doses between 40 and 80 mg/kg usually caused abrupt respiratory arrest followed by cardiovascular collapse and death. Respiratory support frequently prevented death but in a small percentage of animals circulatory collapse proceeded respiratory failure.

In conscious rabbits, esmolol at 0.031 to 3.1 mg/kg/min caused a dose-related decrease in heart rate and blood pressure. The onset of effects occurred in 1-2 min, became stable in 6 min and when the infusion was terminated recovery occurred in 20 min. Similar effects were seen in rabbits treated with methylatropine. In anesthetized rabbits, esmolol, at 1 mg/kg/min, reduced heart rate, blood pressure, cardiac output, total peripheral resistance, and mesenteric arterial resistance but not mesenteric arterial blood flow. Propranolol caused similar effects but did not lower blood pressure and significantly increased peripheral resistance. Propranolol prevented the effects of esmolol on heart rate and cardiac output but not on blood pressure or total peripheral resistance. These experiments indicate that esmolol decreased blood pressure in rabbits by a non-beta-adrenergic receptor mediated vasodilatory mechanism. Under similar conditions propranolol caused vasoconstriction.

In conscious dogs, esmolol at up to 160 mcg/kg/min had no significant effects on heart rate, systolic or diastolic blood pressures, left ventricular end-diastolic pressure, stroke volume, total peripheral resistance, QRS complex, T wave or ST segment but did cause a dose-related decrease in dp/dt max for from -11% at 20 mcg/kg/min to -23% at 160 mcg/kg/min. The highest dose also prolonged the PR interval and reduced pulse pressure. All effects were reversible in 20 min. In anesthetized dogs, however, esmolol at 5 to 160 mcg/kg/min produced dose-dependent decreases in heart rate, diastolic blood pressure, myocardial contractile force, left ventricular dp/dt, the rate-pressure product, and diastolic coronary blood flow; systolic pressure, mean coronary blood flow and mean coronary vascular resistance were not significantly altered. Esmolol did not significantly alter the hyperemic response to 10 sec of coronary occlusion nor the time to 50% recovery from the peak hyperemic response. Esmolol at 310 mcg/kg/min did, however, slightly (20% or less) but significantly decrease coronary blood flow and myocardial oxygen consumption, increase coronary vascular resistance but did not affect systolic or diastolic blood pressure, or myocardial oxygen extraction. It should be noted that esmolol did not always lower blood pressure in anesthetized dogs and when this did occur the effect was generally small.

The hemodynamic effects of esmolol at 100 to 1000 mcg/kg/min in enflurane anesthetized dogs were not substantially different than seen in other studies in pentobarbital anesthetized dogs.

The hemodynamic effect of esmolol at doses less than 2000 mcg/kg/min were prevented by ganglionic blockade with hexamethonium, by catecholamine depletion with reserpine, and by beta-receptor blockade with propranolol. Thus the hemodynamic effects of esmolol at these doses are caused by the reduction of endogenous sympathetic tone due to beta-adrenergic receptor blockade.

Effect of Esmolol on Myocardial Infarct Size:

In anesthetized dogs with a critical coronary stenosis (no reduction of blood flow but abolition of reactive hyperemia), esmolol infused at 50 mcg/kg/min 20 min before complete occlusion, during occlusion (60 min) and for 4 hours post re-perfusion significantly reduced infarct size seen 24 hrs later from 24% of the left ventricle in controls to 7.5%.

The above effect of esmolol was confirmed and extended in a second study in anesthetized dogs. Esmolol (100-150 mcg/kg/min) reduced heart rate before and during coronary artery occlusion, prevented heart rate increases and tachyarrhythmias on reperfusion, prevented the worsening of systolic bulging on reperfusion, and significantly reduced the infarct size. The compound did not depress the non-ischemic zone, alter flow during occlusion or affect the area at risk. The beneficial effect was probably due to a decrease in the oxygen requirement due primarily to a reduction in heart rate and prevention of reperfusion tachycardia. As indicated by the Authors, these data suggest that the agent may be potentially useful in the early treatment of patient with acute myocardial infarction about to undergo early revascularization, either thrombolytic or surgical.

Antiarrhythmic Activity:

In anesthetized dogs, esmolol infused at 150 mcg/kg/min before and during occlusion of the left anterior descending coronary artery reduced the incidence of ventricular tachycardia and prevented ventricular fibrillation.

Electrophysiologic Effects:

In anesthetized dogs, esmolol at 310 mcg/kg/min in unpaced dogs prolonged the P-P interval, and the PR interval but did not significantly affect blood pressure, QRS duration, AH interval or HV interval. During atrial pacing, esmolol prolonged sinus node recovery time, prolonged the stimulus-H interval, and prolonged the AH interval but did not affect the HV interval. Esmolol also prolonged the relative and functional refractory periods of the AV node, and prolonged the Wenckebach cycle length. Recovery occurred in 30 min. These are expected effects of a beta-receptor antagonist.

Local Anesthetic Activity:

Esmolol had no local anesthetic activity in the frog sciatic nerve at up to 33.2 mg/ml or when placed on the rabbit cornea at a concentration of 1%. Esmolol did cause a short lasting slight but incomplete corneal anesthesia at concentrations of 3% and 10%. The maximal effect of esmolol was about equal to 4% lidocaine, a relatively poor corneal anesthetic. Propranolol at 0.1 to 10% caused marked corneal anesthesia and was about 200 times more potent than esmolol.

In the rabbit, esmolol was an effective infiltration anesthetic at concentrations of 0.3% or greater and as such was more potent than lidocaine but its effect was associated with edema which became marked at concentrations above 1%.

Intrinsic Sympathomimetic Activity:

Esmolol had no intrinsic sympathominetic activity in isolated guinea pig atria from catecholamine depleted animals but in isolated rabbit cardiac tissue concentration of esmolol of 10^{-7} to 10^{-4}M tended to increase SA nodal automaticity.

In anesthetized, reserpinized dogs, esmolol at 0.33 to 3.8 mg/kg/min did not have intrinsic sympathomimetic activity.

Direct Cardiac Depressant Activity:

Direct cardiac depression is defined as cardiac depression not due to beta-adrenergic receptor blockade.

In isolated guinea pig atria, depression of spontaneous rate and force of contraction occurred at concentrations of 10^{-4} M and 10^{-3} M, respectively.

In isolated rabbit cardiac tissue esmolol at concentration of 10^{-4}M and above decreased SA nodal automaticity diastolic resting potential, Vmax, action potential amplitude and overshoot. In AV nodal tissue concentrations of 6 X 10^{-5}M or 10^{-4}M slowed conduction and prolonged the effective refractory period, respectively. At 10^{-3}M esmolol caused AV nodal block.

In isolated canine purkinje fibers esmolol at concentrations of 10^{-4}M and above reduced action potential amplitude, Vmax, and action potential duration but the effective refractory period was unaffected. At 10^{-3}M esmolol reduced the resting diastolic potential (depolarized) and the fibers became inexcitable.

Thus the direct depressant effects of esmolol on myocardial electrophysiology of isolated tissues occurs at concentrations 2 to 3 orders of magnitude higher than concentrations causing beta-receptor antagonism.

In anesthetized, catecholamine depleted dogs, esmolol at less than 0.75 mg/kg/min did not have hemodynamic effects. However, at 0.75 or 1.13 mg/kg/min and above esmolol caused dose related reductions of blood pressure, heart rate, contractile force (paced or unpaced) left ventricular dp/dt, and increased left ventricular end-diastolic pressure. The highest dose of esmolol (3.8 mg/kg/min) caused marked hemodynamic depression but on discontinuation of the infusion, recovery started in 1 min and was complete in 20 min. These deressant doses of esmolol are much larger than the beta-receptor blocking dose in the anesthetized dog (0.005 to 0.160 mg/kg/min, with an ID_{50} of 50 mcg/kg/min and a pA_2 of about 20 mcg/kg/min). Propranolol and metoprolol caused similar effects but pacing was frequently not possible after propranolol and recovery from hemodynamic depression caused by large doses did not occur. The ID_{50} against isoproterenol for esmolol, propranolol, and metoprolol were 0.05 mg/kg/min, 0.06 mg/kg, and 0.09 mg/kg, respectively. The relative safety, based on the ratio of the dose that caused a 50% recuction of contractile force (DD_{50}) to the isoproterenol ID_{50} dose, for esmolol, propranolol and metoprolol was 33, 433 and 129-306.

levo-Esmolol vs dl-Esmolol:

In isolated guinea pig atria, levo-esmolol was slightly less potent as a beta-receptor antagonist than dl-esmolol. In the anesthetized dog both levo- and dl-esmolol caused dose-related decreases in heart rate but neither altered blood pressure. The levo- isomer was about 2X more potent than the racemate (dl-esmolol) as a beta-receptor antagonist. Neither compound inhibited the hypotensive effect of isoproterenol even at up to 113.9 mcg/kg/min. Thus, levo-esmolol is responsible for most, if not all, of the beta-adrenergic receptor blocking activity of the racemate.

In isolated guinea pig atria, levo-esmolol caused concentration-related decreases in both the spontaneous right atrial rate and the force of left atrial contractions with 50% Depression Concentrations (DC_{50}) of 1.74×10^{-4} M and 8.7×10^{-4}M, respectively; dl-ASL-8052 was about 2X more potent in depressing spontaneous rate ($DC_{50} = 9.6 \times 10^{-5}$M) but about equipotent in depressing force of contraction ($DC_{50} = 9.8 \times 10^{-4}$M).

Effects of ASL-8123, the Acid Metabolite of Esmolol:

ASL-8123, the primary acid metabolite of esmolol was a competitive, cardio-selective beta-receptor antagonist which in vitro (guinea pig atria) and in vivo (anesthetized dog) was less potent that ASL-8052 by factors of 350 fold and 320 fold, respectively.

In the rabbit, the acid metabolite of esmolol, ASL-8123 , did not cause corneal anesthesia at concentration of up to 10%. It did cause slight infiltration anesthesia at concentration of above, but not below, 1%. Thus the metabolite was less active than the parent compound as both a surface and an infiltration anesthetic.

Miscellaneous Effects:

In conscious rabbits, 3% esmolol applied to one eye reduced the glucose-induced (i.v. adminstration) increase in intra-ocular pressure in both the treated and contralateral eye at 1 but not 4 hours. These data are compatible with systemic absorption of esmolol after ocular administration as demonstrated in pharmacokinetic studies.

In anesthetized, vagotomized, propranolol-treated dogs, esmolol at 100 or 300 mcg/kg/min (i.e., 2-6 times the isoproterenol ID_{50}) did not modify the bradycardia induced by submaximal vagal stimulation.

PHARMACOKINETICS:

In Mongrel dogs the i.v. infusion of esmolol at 25, 50 and 100 mcg/kg/min caused dose-related increases in steady state blood levels of esmolol of 0.09, 0.20 and 0.46 mcg/ml, respectively, and dose-related inhibition of heart rate responses to a standard dose of isoproterenol of 43%, 59% and 69%, respectively. Steady state blood levels were obtained within 30 minutes and maximal beta blockade was achieved within 10 min of initiating each infusion. Blood levels in these mongrel dogs were more variable and slightly lower (19% to 34%) than in a smaller group of beagle dogs which received the same doses of esmolol. These differences in blood levels were not reflected in differences in beta-receptor antagonism based on the % inhibition of isoproterenol.

Blood levels of esmolol decreased very rapidly after stopping the infusion and reached levels of less than 10% (below detectable limits) of steady state levels within 15 min in all dogs. It appeared that esmolol disappearance more slowly from mongrel than beagle dogs, however, this difference was not certain since blood samples were obtained from only a few animal of each strain during recovery. Recovery from beta-receptor blockade was also very rapid with full recovery in most animals in 20 min. The few animals not fully recovered in 20 min were mongrel dogs.

In conscious mongrel dogs given a bolus injection of esmolol (10 mg/kg) the blood levels of parent compound disappearance biexponentially according to a two-compartment model. The acid metabolite, ASL-8123, disappearance monoexponentially, consistent with a one-compartment model. When esmolol was infused at a constant rate of 200 mcg/kg/min, steady state plasma levels of 0.632 $\pm$0.2 mcg/ml were attained within 20 min. This is in agreement with the blood levels seen in mongrel dogs infused at 25, 50 and 100 mcg/kg/min of esmolol

The elimination half-life of esmolol in mongrel dogs after bolus (51 min) or infusion (41 min) was 4 to 5 times longer than that reported in humans (9.19 $\pm$ 3.51 min).

Renal clearance of unchanged compound was only about 0.5% of total body clearance indicating extensive and rapid metabolism in the dog. In support of this, only about 0.5% of the dose was recovered in the urine as unchanged esmolol while 57-69% of the dose was recovered as the metabolite, ASL-8123.

The elimination of the metabolite, ASL-8123, was much slower (t1/2: 106-112 min) than that of the parent compound (longer elimination half-life, slower total body clearance and much greater AUC). In addition, peak plasma levels of the metabolite did not occur until about 30 min after the bolus administration and 6 min after the termination of the infusion.

The total body clearance of esmolol was 10 times the reported normal hepatic blood flow in dogs (37.5 ml/kg/min). The sponsor reports a similar finding in humans where total body clearance was 20 times hepatic blood flow. These data suggest that the liver does not play an important role in the metabolism of esmolol. This conclusion is supported by the observation in dogs that shunting blood past the liver just prior to terminating a 3 hour infusion of esmolol did not prolong the duration of beta-receptor antagonism.

The volume of distribution of the parent compound was 34 to 46 times larger than the reported total body water in dogs (0.6 l/kg). This coupled with the observation that the parent compound is not extensively bound to plasma proteins and the volume of distribution is larger than the central compartment indicate preferential tissue uptake of the compound.

Peak plasma levels of the metabolite were 40 times higher than levels of the parent compound. In addition, the volume of distribution of the metabolite was much smaller than that for the parent compound and only 1.3 to 1.7 times total body water (0.6 l/kg).

In beagle dogs infused with esmolol at 50 to 1000 mcg/kg/min for 5 days or at 100 to 800 mcg/kg/min for 2 weeks there was a dose related increase in plasma levels of esmolol and ASL-8123, there was no apparent accumulation of either compound, there was no apparent sex difference in plasma levels, and levels of ASL-8123 always greatly exceeded levels of esmolol (17 to 113 X).

In Sprague-Dawley rats esmolol was not found in large amounts in any tissue. Within 5 min of administration of 14C-esmolol, labeled material (which included both parent compound and metabolite) was found in all tissues and organs examined including blood; peak levels occurred in 5 to 15 min. Amounts ranging from 2% to 9% of the administered label were found (arranged from highest to lowest) in the liver, GI tract , kidneys, blood, and lung . Less than 1% of the labeled material was found in the heart, brain, spleen and testes. The remaining carcass plus the skin contained about 59% of the administered dose. At 5 and 10 min 88.1% and

87.5% of the doses, respectively, was accounted for. This declined to 14.9% at 420 min (7 hrs) indicating that about 85% of the dose had been eliminated (urine, feces, etc) by the animals. In all tissues and at all time points, most of the labeled material was the acid metabolite (ASL-8123) of esmolol indicating rapid hydrolysis. Thus, the ratio of acid metabolite:esmolol was greater than 1 in all tissues and reached very high levels in the kidneys and liver. This could indicate that the kidney and liver were major sites of metabolism of esmolol or that these tissues have a high affinity for the metabolite generated by hydrolysis of esmolol in blood. It should be noted that in other studies in the rat 80% of administered esmolol was excreted by the kidney as the acid metabolite and less than 5% of the parent drug was recovered in the urine. The tissue:blood ratio for esmolol at all time points was highest for brain and greater than 1 in other tissues except in the kidneys and liver. In contrast, the tissue:blood ratio of the acid metabolite was greater than 1 in all tissues and was highest in kidney and liver. Esmolol rapidly entered the brain achieving concentrations that were more than 10 times higher than blood levels. In contrast, the levels of the acid metabolite in brain and blood were about equal, although levels of the acid metabolite in the brain were higher (about 2 X) than levels of esmolol. The blood and tissue concentrations of esmolol were lower than those of its metabolite indicating rapid hydrolysis. The rate of elimination of esmolol from blood and tissues was rapid with a half-life of from 7 to 12 min and followed first order kinetics. The elimination from blood, kidney and liver was about equal and faster than in brain and heart. Based on both half-lives and AUCs the rate of elimination of the acid metabolite from blood and tissues was much slower than for esmolol.

In the rat total urinary and fecal excretion of labeled material 45-47 hours after administration of 14C-esmolol as either a single bolus (30 mg/kg), injections (12 mg/kg) every 2 hours for 10 hours, or by infusion (100 mcg/kg/min) for 10 hours ranged from 93-101%. The similarity of recovery with single, multiple doses, and infusion indicates the enzyme system responsible for the hydrolysis of esmolol was not saturated by the multiple dosing or infusion schedules. However, saturation of the enzyme system may have been responsible for the lower ratio of plasma ASL-8123:esmolol at esmolol doses of 40 mg/kg/min than at 5 mg/kg/min for 2 weeks.

The protein binding of esmolol or its acid metabolite was not extensive in the mouse, rat, rabbit, dog or human. The serum protein binding of esmolol at a concentration of 6.6 mcg/ml was greatest in human (56%) and mouse (56%) and slightly greater than in rat (44%), rabbit (42%), or dog (38%). The protein binding of its acid metabolite (ASL-8123) was much less than for the parent compound ranging from 1% - 2% in rat, rabbit and dog serum to 7% and 9% in human and rabbit serum, respectively. In human serum the binding of esmolol and its metabolite was about equally divided between albumin and a_1-acid glycoprotein (AAG). Neither Esmolol at 3.28 to 127.6 mcg/ml (9.9 to 385 mcM) or ASL-8123 at 35.5 to 355 mcM demonstrated concentration-dependent binding to AAG (13 to 26% bound) or rat serum (8.5% to 15.9% bound), respectively.

Esmolol added in vitro to blood from rats, guinea pigs, rabbits, dogs and humans was hydrolyzed with half-lives of less than 2.5 min for rats and guinea pigs, 8 min for rabbits, 8-15 min for dogs, and 27-30 min for humans. Thus, the hydrolytic of activity was highest in whole blood from rats and guinea pigs and lowest in whole blood from humans. In addition, most of the esmolol esterase activity in rats and guinea pigs was found in the plasma but in dog and man it was in the erythrocyte. In dog blood, the in vitro half-life of esmolol was independent of substrate concentration suggesting that hydrolysis of esmolol follows 1st order kinetics which is in agreement with in vivo studies. Esmolol esterase activity was not found in dog plasma or erythrocyte membranes but in the erythrocyte cytosol (lysate). Although plasma had no activity alone or when combined with erythrocyte membranes, it did enhance the activity of erythrocytes and erythrocyte cytosol. The buffering capacity of plasma may play a role in its ability to enhance the esterase activity of erythrocytes. The Sponsors expanded summary (Table 2, p 47) indicates that for full activity dog erythrocyte cytosol requires a heat labile, high molecular weight (greater than 300K) plasma component. The source of these data was not stated nor were they found in the submitted studies.

The differences in activity and location of the esterase responsible for the hydrolysis of esmolol mentioned above suggests that the esterase in dog and man are similar but different than the esterase in rat and guinea pig. This is supported by the observation that while NaF inhibited esmolol esterase activity in blood from rats, guinea pigs, dogs and humans, echothiophate inhibited esmolol esterase activity of blood from rats and guinea pigs but not from dogs and humans. Eserine did not inhibit esmolol esterase activity of blood from any of the 4 species, indicating that it is different than acetyl- or pseudo-cholinesterase.

Esmolol esterase activity was not restricted to blood since in vitro studies showed that ocular tissue from rabbits also hydrolyzed esmolol. Esmolol esterase activity was present (in decreasing order) in the iris-ciliary body, cornea, sclera and lens but not in aqueous or vitreous humor and esmolol half-lives ranged from 8 to 62 min. Tissues of the rabbit eye had fast and slow esterase activity but the Sponsor did not indicate whether the rate of hydrolysis of ASL-8052 varied with fast and slow ocular esterase activity.

There was no difference in the esmolol esterase activity between human female and male whole blood. In addition, unlike found in a rat teratology study, blood from human females did not indicate the presence of "fast" and "slow" esmolol esterase activity.

To further characterize esmolol esterase, in vitro studies indicated that the following purified enzymes or blood proteins failed to hydrolyze esmolol: dog, chicken, rat or human albumin; human serum pseudocholinesterase; human hemoglobin; human erythrocyte carbonic anhydrase A or B; human or bovine erythrocyte acetylcholinesterase; and electric eel acetylcholinesterase.

The above indicates that esmolol esterase is specific and not one of the well known esterases. However, several experiments were performed to determine the potential interaction of esmolol with other drugs metabolized by esterases.

The metabolism of esmolol by dog or human whole blood was not inhibited by acetylcholine, succinylcholine, procaine or chloroprocaine. Like wise, esmolol did not alter the metabolism of either procaine or chloroprocaine by dog or human whole blood.

In dogs, succinylcholine had no effect on the beta-receptor blocking activity or the duration of activity of esmolol infused at 50 mcg/kg/min for 3 hours. In other studies with mongrel dogs, this infusion rate of esmolol resulted in steady state blood levels of 0.2 mcg/ml, was equal to the isoproterenol ID_{50} dose, and was about 2.5 times the in vivo pA_2 for isoproterenol. Furthermore, in contrast to eserine, the infusion of esmolol at doses up to 300 mcg/kg/min failed to prolong recovery from succinylcholine-induced respiratory paralysis.

At concentrations of 0.5 to 2 mcg/ml esmolol had little or no effect on the hydrolysis of benzoylcholine by human plasma or whole blood. In addition, concentrations up to 4.2 mcg/ml had little effect on the whole blood half-life of benzoylcholine and caused only a 12% inhibition of hydrolysis by plasma. Higher concentrations caused a concentration-dependent inhibition of benzoylcholine hydrolysis with an ID_{50} of 30 mcg/ml. Benzoylcholine, like succinylcholine, is readily metabolized by plasma cholinesterase (pseudocholinesterase). This concentration is 20 times and 2.4 times that seen in beagle dogs infused at 800 mcg/kg/min and 1000 mcg/kg/min, respectively.

Thus, neither acetylcholinesterase (located in erythrocyte membranes) or pseudocholinesterase (buterylcholinesterase) (located in plasma) are responsible for the hydrolysis of esmolol. Esmolol esterase is located in the cytoplasm of erythrocytes but is not hemoglobin, carbonic anhydrase or an esterase that is inhibited by eserine. As pointed out by the sponsor, the esterase may be similar to aspirin esterase which is also located in the cytosol of human erythrocytes and is not inhibited by eserine. Failure of either echothiophate (organic phosphate) or eserine to inhibit dog or human esmolol esterase suggests that it may be an arylesterase (located in the erythrocyte cytosol). Esmolol should not interact with other esters which are metabolized by acetylcholinesterase or plasma cholinesterase as demonstrated for procaine, chloroprocaine, acetylcholine and succinylcholine.

The intended route of administration of esmolol is intravenous; however, the compound is absorbed orally or when instilled into the eye.

In the rat the oral LD_{50} (8.9 gm/kg) was approximately 125 times the i.v. LD_{50} (58.71 mg/kg) and at least 180 times the i.v. dose causing behavioral effects (prostration). Thus, oral esmolol is either rapidly hydrolyzed or poorly absorbed.

In both the rabbit and dog esmolol instilled into the eye is rapidly absorbed not only into structures of the eye but systemically. In the dog esmolol was detected in the plasma in 30 sec and reached peak levels in less than 2.5 min in rabbits and 5 min in dogs. In the rabbit the area under the blood-time curves of both esmolol and ASL-8123 indicate that about 17% of the ocular dose was absorbed systemically. In the dog the systemic bioavailibility (based on i.v. and ocular AUCs) of esmolol after ocular administration was estimated to be 1.9% and that of ASL-8123 was about 35%. These are only estimates since loss of drug due to tearing and overflow were not accounted for. It should be noted that systemically administered esmolol gains access to eye structures but in low concentrations.

TOXICOLOGY:

Acute Toxicity:

The single dose i.v. LD_{50}s of esmolol in mice, rats, rabbits and dogs were approximately 93, 71, 40 and 32 mg/kg, respectively. Signs of toxicity included dyspnea, prostration, clonic/tonic convulsions, hypokinesia, hyperpnea, ataxia, sedation and tremors. The acute toxicity of the compound in the dog was greatly reduced when administered slowly. Thus, when administered over a 1 hour rather than a 1 min interval, no behavioral effects were seen at up to 120 mg/kg, while 180 mg/kg caused only salivation and some ataxia; no deaths occurred at up to 240 mg/kg (7.5 times the LD_{50}). In mice the LD_{50} for ASL-8123, the acid metabolite of esmolol, was nearly 7 times higher than that for the parent compound. In addition, methyl alcohol (80 mg/kg, i.v.), a metabolic by-product of esmolol, was nontoxic at a dose of 1.3 times that expected at an ASL-8123 dose of 582 mg/kg.

Sub-Chronic Toxicity:

In a two week i.v. rat study esmolol was toxic at 40 mg/kg/day causing ataxia, reduced motor activity, prostration and respiratory distress; 4 out of 30 rats died. No gross or microscopic pathology was associated with esmolol treated rats that died during test or survived to study termination. The mid-dosage level (20 mg/kg/day) also caused reduced motor activity, ataxia and respiratory distress in several animals. The low dose (5 mg/kg/day) had no effects.

In sub-chronic i.v. dog studies, esmolol caused no mortalities at up to 1000 mcg/kg/min for 5 days or up to 800 mcg/kg/min for 14 days. The 1000 mcg/kg/min infusion rate produced severe pharmacologic effects including convulsions, prostration, ataxia, emesis, head tremors, and muscle rigidity. No effects were observed at 50, 200 or 400 mcg/kg/min while 500 mcg/kg/min induced hyperexcitability, head tremors and muscle rigidity; 800 mcg/kg/min for 14 days caused emesis and reduced motor activity. Food consumption was decreased 10 to 18% in a dose-related manner. At up to

800 mcg/kg/min administered for 14 days esmolol had no effects on ECG, the eye, on urinalysis (note, both esmolol and ASL-8123 gave false positive for urine protein) or on gross or histopathology. Changes in clinical chemistries and hematologic parameters were seen in females and consisted of a dose-related decrease in hemoglobin and hematocrit at 100, 400 or 800 mcg/kg/min and a decrease in RBC's in the two high dose groups; there was also a slight elevation in BUN at termination. All values were within normal limits and not associated with gross or microscopic pathology.

Chronic Toxicity: These studies were not performed or required for the intended short term clinical use or esmolol.

Mutagenicity Studies: None submitted or required.

Reproduction Studies: The sponsor, at our request, performed Segment II Teratology studies in the rat and rabbit. Because of the intended clinical used of the compound Segment I and III reproduction studies were not considered necessary.

Teratology Studies:

In the rat intravenous infusion of ASL-8052 at up to 3.0 mg/kg/min for 30 min a day on days 6 through 15 of gestation was not maternotoxic, fetotoxic, or teratogenic. Infusion of 10 mg/kg/min caused excessive maternal mortalities (probably due to excessive pharmacodynamic effects) but was not fetotoxic or teratogenic.

In a rabbit teratology study esmolol was infused i.v. at 0.5, 1.0 or 2.5 mg/kg/min, 24 hrs a day on days 6 through 18 of pregnancy. There was a dose-dependent increase in peak blood levels of both ASL-8052 and its major acid metabolite, ASL-8123. Plasma levels of ASL-8123 always exceeded levels of esmolol by 5 to 20 times. None of the clinical and necropsy findings in animals that died or survived to sacrifice appeared drug-related but appeared related to the trauma of the treatment procedure. Findings were compatible with spinal cord injury and systemic infection. During the treatment period (days 6-18), does in the 2.5 mg/kg/min group lost weight resulting in a significantly lower weight gain during gestation (day 0-19) than in the other groups. Esmolol at 2.5 mg/kg/min significantly increased post-implantation losses, primarily by increasing early resorptions. The compound had no effect on late resorptions, the incidence of non-viable fetuses, or the average fetal weight of viable fetuses. There were no drug-induced increases in the incidence or number of malformations, nor was there an increase in developmental variations. Therefore, in the rabbit, ASL-8052 administered on days 6 through 18 of pregnancy at 1.0 mg/lkg/min X 30 min/day was not toxic to the dams, embryos, or fetuses. At 2.5 mg/kg/min X 30 min/day (75 mg/kg/day) esmolol caused some maternal toxicity and increased early fetal resorptions but was not teratogenic.

Compatibility with Human Blood:

The sponsor determined the blood compatibility of esmolol either as a 10% solution (100 mg/ml) in an acetate buffered 10% propylene glycol-10% alcohol formulation, a 10% solution in an acetate buffered 70% propylene glycol-30% alcohol formulation, a 25% solution (250 mg/ml) in acetate buffered 25% glycerin-60% alcohol formulation, or a 25% solution (250 mg/ml) in acetate buffered 0.9% Saline.

In human plasma, the 10% esmolol formulation did not cause significant plasma protein flocculation and, with saline dilutions, there was no significant hemolysis at esmolol concentrations up to 5 mg/ml. Higher concentrations caused hemolysis the degree of which was concentration-dependent. The maximal hemolysis at an esmolol concentration of 100 mg/ml was about 70%. Similar results were obtained with the 10% esmolol solution in the 70% propylene glycol-30% alcohol formulation.

A 1:50 saline dilution (5 mg/ml) of the 25% esmolol formulation was the highest concentration that did not produce substantial hemolysis or plasma protein flocculation in human blood. Concentrations of 20 mg/ml to 250 mg/ml (non-diluted formulation) produced 52% to 77% hemolysis while concentrations of 100 and 250 (non-diluted) mg/ml produce 8% to 9% protein flocculation. The degree of protein flocculation was substantially less than the 100% seen with the positive control (TEA). The hemolysis and plasma protein flocculation produced by this formulation were similar to those produced by equal concentrations of the 10% ASL-8052 formulation mentioned above.

When compared to vehicle, addition of undiluted 25% esmolol in acetate buffered saline to 4 parts of test material (final concentration = 50 mg/ml) did not affect clot retraction or platelet adhesiveness but did prolong partial thromboplastin time, prothrombin time, and thrombin time and may have slightly decreased fibrinogen concentrations.

Acute Toxicity of Esmolol Formulations:

In the rat, the sponsor examined the the acute toxicity of a 5% esmolol solution (50 mg/ml) in an acetate buffered 10% propylene glycol-10% alcohol formulation, a 10% esmolol solution (100 mg/ml) in an acetated buffered 10% propylene glycol-10% alcohol formulation, and a 25% esmolol solution (250 mg/ml) in a 20% glycine-60% alcohol formulation. The signs of toxicity were essentially the same with each esmolol formulation and the LD_{50}s ranging from 57 to 71 mg/kg were not substantially different than seen when esmolol was dissolved in saline.

Vascular and Perivascular Irritation:

In the rabbit ear i.v. infusion or perivascular injection of undiluted 10% esmolol (100 mg/ml) in a 70% propylene glycol-10% alcohol formulation caused an intense and extensive inflammatory and necrotizing reaction with little or no resolution in 10 days. A 1:30 dilution (33 mg/ml) was slightly irritating and a 1:100 dilution (1 mg/ml) was no more irritating than saline.

Additional local irritation studies in the rabbit ear indicated that esmolol either as a 10% solution in 10% propylene glycol-10% alcohol or as a 25% solution in 20% glycerin-60% alcohol produced substantial venous irritation at concentrations as low as 5 mg/ml (lowest concentration tested). At equal or similar concentrations the 25% formulation produced significantly more venous and perivascular irritation than the 10% formulation suggesting that the glycerin (20%)-alcohol (60%)-water vehicle was more irritating than the propylene glycol (10%)-alcohol (10%)-water vehicle.

Intravenous infusion or perivascular administration of undiluted vehicle (acetate buffered 10% Propylene Glycol- 10% Alcohol) also caused substantial irritation. This irritation was eliminated when the vehicle was diluted with saline as little as 1:5. When administered i.v., esmolol at concentrations of about 10 mg/ml or greater in either saline or vehicle (1:10 dilution) caused a concentration-dependent irritation (no significant difference between preparations) that ranged from slight to severe. The esmolol formulation, however, tended to produce slightly more irritation than esmolol in saline when administered perivascularly. These results indicate that esmolol itself is the main cause of local vascular irritation produced by intravenous infusion. This irritation is manifested in perivenous hemorrhage, perivenous edema, perivenous inflammatory infiltrate, occluding and non occluding thrombi, leucocyte pavementing, and necrosis.

The local irritation seen with i.v. infusions in the rabbit ear depends on the rate of infusion since when the 10% esmolol solution (100 mg/ml) in the acetate buffered 10% Propylene Glycol-10% Alcohol formulation was diluted with saline to a final concentration of 10 mg/ml and infused at rates of 0.2 to 10 mg/min substantial venous irritation was seen only at infusion rates of 2 mg/min or higher.

Studies in the dog indicate that infusion of undiluted 10% esmolol in an acetate buffered 10% propylene glycol-10% alcohol formulation at 500 mcg/kg/min for 3 days caused mild to severe local irritation characterized by mild to severe thrombophlebitis with extension of inflammation into surrounding tissues.

Additional i.v. studies in dogs indicate that infusion at 300 mcg/kg/min of the 10% esmolol solution in the acetate buffered 10% propylene glycol-10% alcohol formulation for 24 to 72 hours even when diluted to a concentration of 10 mg/ml (1:10) with 5% dextrose caused slight to severe thrombophlebitis with occasional occlusion of the infused vein. The incidence and severity of these findings increased with increasing infusion durations. The Sponsor states "Lung thrombi occurred in one or two dogs from each group. There was no relationship between occurrence of lung thrombi and material infused or duration of infusion. All thrombi observed occurred in vessels less than 0.5 mm diameter and there was no evidence of infarction associated with thrombi". It is not clear whether these pulmonary thrombi represent emboli from the cannulated vein or arose during sacrifice (anesthesia and exsanguination), but absence of infarction suggests the latter.

Since methy ethyl ketone is a slight contaminant (111 ppm) in the 10% esmolol formulation the sponsor attempted to determine its role in local irritation. In dogs, infusion of normal saline or methy ethyl ketone in saline into a cannulated jugular vein for 72 hours produced a non-inflammatory reaction to mild thrombophlebitis, probably due to mechanical irritation of the catheter. Infusion of esmolol (300 mcg/kg/min) either in saline or in its final formulation, caused severe irritation characterized by extensive thrombophlebitis and necrosis of the vein wall with frequent extension of the inflammation, and occasionally necrosis, to the surrounding tissue. Although the venous irritation may have been more severe with the esmolol formulation than with the esmolol in saline, it is clear that the major cause of the irritation was esmolol and not the vehicle or the methy ethyl ketone contaminant. There was no evidence of esmolol-induced pulmonary lesion which might result from increased numbers of emboli.

Acute Toxicity of levo-Esmolol:

The sponsor compared l-esmolol with dl-esmolol in terms of acute toxicity and the local irritating activity.

In mice, the acute i.v. toxicity of l-esmolol was essentially the same as the racemate. In rats, the acute i.v. toxicity of l-esmolol and dl-esmolol were similar but the levo-isomer tended to be slightly less toxic and caused a higher incidence of sedation than the dl-isomer. In the dog, i.v. infusion of l-esmolol or dl-esmolol over a 1 hour interval at increasing concentrations caused essentially the same effects. Thus, 2000 mcg/kg/min was the maximum tolerated dose and 3000 mcg/kg/min produced salivation, tremors, ataxia, sedation, hyperpnea and death. Terminating the infusion of high doses (4000 mcg/kg/min) prevented death in about half of the animals. In animals which survived, recovery occurred within 5 min.

In local irritation studies in the rabbit ear, microscopically, both the l- and dl-forms of ASL-8052 produced significantly more venous and perivascular irritation 24 hours after a 6 hr i.v infusion or a single perivascular injection, respectively, than saline at concentrations or 10 mg/ml and above. There was a suggestion that the l-form was slightly less irritating than the dl-form but this was not conclusive. No venous or perivascular irritation was observed in saline-treated rabbits or those treated with either l- or dl-ASL-8052 at a concentration of 5 mg/ml delivered at 1.2 mg/min.

Acute Oral Toxicity and Ocular Toxicity:

Although not bearing directly on the clinical use of esmolol as an intravenous product, for completeness, these are summarized.

Acute Oral Toxicity: In rats, single oral doses of ASL-8052 caused no observable effects at up to 1 gm/kg and no deaths at up to 4 gm/kg. The only effect seen at non-lethal doses was sedation. The oral LD50 (8.91 gm/kg) was approximately 125 times greater than the i.v. LD50 (10.9 mg/kg) in rats. These results suggest that oral ASL-8052 is poorly absorbed and/or rapidly metabolized.

Ocular Toxicity: Esmolol at 1% in saline or in an acetate buffer (plus 0.01% benzalkonium chloride) did not produce immediate ocular irritation in the rabbit. In contrast, esmolol at 3% and 10% either in saline or the acetate buffer caused slight and moderate immediate irritation, respectively, characterized by conjunctival erythema, chemosis, and discharge. These effects lasted less than 10 minutes. Sub-chronic (t.i.d. for 6 days) ocular administration of esmolol at 0.5%, 1.0% or 3.0% was not irritating nor did it cause gross of histologic ocular pathology.

The ocular irritation produced by the 3% and 10% acetate buffer formulations of esmolol was qualitatively similar, but slightly greater than that produced by the same concentration of esmolol dissolved in saline. The acid pH of the acetate buffer formulations can not account for the enhanced irritation since the pH of both the vehicle alone or the 1% formulations were similar (about 4.7). The enhanced irritation is not likely due to solution osmolarity since the osmolarity of the 3% acetate formulation was similar to that of saline. The 10% acetate formulation was, however, hyperosmolar. The Sponsor believes that the irritation produced by the 3% and 10% acetate formulation was a result of potentiation between vehicle and drug.

VI. RECOMMENDATIONS:

Based on non-clinical studies, esmolol in approvable for the intended clinical use.

The submitted Package Insert is satisfactory with regard to non-clinical portions.

for Gordon L. Johnson, Ph.D.
Pharmacologist
date 5/1/86

cc: Orig. NDA 19-386
HFN-110
HFN-110/CSO
HFN-102/VGlocklin
HFN-345/G[illegible]es
HFN-110/GLJohnson
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